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George P. Fitzgerald, et al

Wisconsin University

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16. Abstract

The availability and utilization of nutrients by algae and rooted aquatic plants was measured in laboratory studies using both sorption and algal growth assays. The tests were conducted to evaluate contributions of phosphorus to the Madison (Wisconsin) lakes from septic tanks, agricultural runoff, and urban drainage and to measure changes in the nutritional status of six lakes which were manipulated for water quality improvement by nutrient inactivation or hypolimnetic aeration. The assay techniques are described and results are compared with chemical determinations of plant nutrients. Comparative rates of phosphorus sorption by different types of aquatic plants were determined by three methods in order to understand how the plants compete for limited phosphorus sources. In situ and laboratory experiments indicated that naturally layered lake muds in three lakes release soluble phosphate-phosphorus when disturbed and ~~which~~ will be competitively sorbed by both muds and plants in aerobic conditions. Studies with duckweed, Lemna minor, showed that it cannot utilize the phosphorus of aerobic lake muds but can absorb phosphorus from lake muds when ~~their~~ its roots are able to penetrate presumably anaerobic areas. Comparisons have demonstrated that ground waters and some storm sewer discharges contain phosphorus compounds that are not available to algae. (Uttormark-Wisconsin)

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APPLICATIONS OF GROWTH AND SORPTION ALGAL ASSAYS

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ABSTRACT

The availability of nutrients in selected Wisconsin lakes was measured in laboratory studies utilizing both sorption and growth algal assays. These tests were conducted to evaluate contributions of phosphorus to the Madison area lakes from septic tanks, agricultural runoff, and urban drainage and to measure changes in the nutritional status of six lakes which were manipulated for water quality improvement by nutrient inactivation or hypolimnetic aeration. Characteristics of the assay techniques are discussed and results are compared to chemical determinations of plant nutrients.

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SECTION I

SUMMARY AND CONCLUSIONS

APPLICATION OF ALGAL ASSAYS TO INLAND LAKE RENEWAL PROJECTS

Chemical analyses of algae and water - Total P and Fe analyses have been selected and evaluated.

Evaluation of algal biomass measurements - Data are presented comparing the utilization of the different methods and for converting the more economical techniques to dry weights.

Analyses of nutrients by algal growth - In excess of 300 lake water samples have been tested with the AAP. In addition, comparisons are given of results obtained with *Selenastrum*, *Microcystis*, *Anabaena*, and the duckweed, *Lemna minor*.

Analyses of available phosphorus by one-day sorption tests with algae - Eight different algae have been successfully used to demonstrate the economy of this technique. This test uses analyses of the P absorbed by algae rather than waiting for a week or more for the algae to grow. The availability of the soluble PO_4-P of lake waters is contrasted to the unavailability of some total P compounds. In addition, the rates of P sorption by different algae have been studied.

Nutritional status of *in situ* algae - The value of these tests was demonstrated when the soluble PO_4-P of lake waters was found to be hardly detectable in mid-summer, but analyses of *in situ* algae indicated that Lakes Monona and Waubesa had significantly higher levels of P available to the algae than Lakes Mendota and Wingra. The algae demonstrate changes in the content of P in lakes that do not appear as surplus P in the water. Similar results have led to laboratory studies of the availability of the P of various lake muds.

RATES OF PHOSPHORUS UTILIZATION BY ALGAE AND AQUATIC WEEDS

Comparative rates of P sorption by different types of aquatic plants have been determined by three methods in order to understand how the plants may compete for limited P sources.

Sorption and growth - Plants are exposed to sources of P for relatively short periods, washed, and subcultured in P-free medium for 1-4 weeks. This technique has been used with *Selenastrum* and the duckweed, *Lemna minor*. Results indicate that maximal amounts of P are sorbed by *Selenastrum* in less than 2 hours, whereas duckweed requires 4 hours. Either plant can utilize pyrophosphate as rapidly as orthophosphate, but tripolyphosphate requires slightly longer exposures.

Analysis of medium *versus* time - Approximately 100 mg of algae are added to media or lake water containing 0.4 mg P/l. Analyses of the supernatant for remaining P are taken for 1 to 6 hours. Results indicate that P-limited *Cladophora* sorb PO_4-P at a rate of 0.05 mg/100 mg algae/hour, whereas *Cladophora* with adequate P sorb P at a rate of 0.02 mg/100 mg algae/hour. The rates for *Rhizoclonium* and *Gloeotrichia* were 0.07 and 0.05 mg/100 mg algae/hour, respectively. In contrast, the rates for duckweed (*Lemna minor*) and tips of *Myriophyllum* sp were 0.003 and 0.008 mg/100 mg/hour, respectively.

Increase in extractable PO_4-P or total P - Duplicate 5 mg samples of algae are added to 1,000 ml media with different levels of P. After 24 hours incubation the extractable PO_4-P or total P of the washed algae is measured. The minimum detectable available P concentration is about 0.02 mg P/l. These techniques can readily be used with mixtures of algae in the same medium. Results when *Cladophora* and *Gloeotrichia* are mixed indicate the algae compete for limited quantities of P, but do not exclude each other.

THE RELEASE, SORPTION, AND AVAILABILITY TO ALGAE OF PHOSPHORUS FROM LAKE MUDS

Experiments carried out *in situ* and in the laboratory indicate that naturally layered lake muds in three lakes release soluble PO_4-P when disturbed. However, when these muds are suspended under the aerobic conditions of the overlying waters, they sorb the phosphorus (P) released within about 20 minutes. The significance of these data is that P released from disturbed muds will be competitively sorbed by both muds and plants.

It was shown that the supernatant waters of a dredging operation contained 0.13 mg soluble PO_4-P/l. However, the *in situ* suspended fine grey sand-to-silt sorbed 50% of the soluble PO_4-P per day.

Samples of *in situ Cladophora* sp from 2 lakes, collected after wind storms had stirred up considerable mud, were found to be P-limited even though the surrounding waters contained as much as 0.8 mg total P/l. It was also shown that *Spirogyra* sp that had grown through layers of dredged mud containing 0.09% P were P-limited.

Laboratory tests of the availability of the P of lake muds under aerobic conditions indicate that *Selenastrum capricornutum* (PAAP) cannot grow on such sources of P, and the N_2-fixing capacity of P-limited *Anabaena flos aquae* (Ind 1444) is not benefited by the P from lake muds. Overnight incubation of P-limited *Cladophora* sp in Lake Mendota water supplemented with both soluble PO_4-P and total P from muddy Crawfish River water indicated, by increases in the total P content of the algae, that it is only able to use the soluble PO_4-P of the Crawfish River water.

Other studies with the duckweed, *Lemna minor*, have also shown that this aquatic weed cannot utilize the P of aerobic lake muds but can absorb P from lake muds when their roots are able to penetrate presumably anaerobic areas. Therefore, it would appear that the growth of rooted aquatic weeds would dominate a eutrophic aquatic environment from which the soluble PO_4-P was removed.

DUCKWEED (*LEMNA MINOR*) FOR NUTRITIONAL BIOASSAYS

Duckweed (*Lemna minor*), a rooted aquatic plant which can be readily cultured under different environmental conditions, has been used in nutritional bioassays. Short-term exposures for sorption in conjunction with 3- to 4-week incubations for growth have been used to show how labile compounds can be tested as available sources of essential nutrients. Tests indicated that ortho- and pyrophosphate could be sorbed in 4 hours while 6 hours were required to sorb tripolyphosphate. Tests under similar conditions indicated that between 6 and 28 hours were required for duckweed to sorb maximal amounts of either NH_4-N or NO_3-N. Tests with Fe as $FeCl_3$ alone or in the presence of citric acid or EDTA indicated that only with EDTA present was the Fe available. Significant sorption of Fe took place with 8-hour exposure when EDTA was present.

Growth experiments lasting 3 weeks in which the availability of the N, P, or Fe of aerobic lake muds was tested indicated that duckweed could not obtain either available N or P under these conditions, but could utilize Fe from the muds.

Comparative tests showed that duckweed could obtain available P from anaerobic lake muds if their roots could penetrate the mud but not if they were suspended out of reach of the mud surface. This indicates how aquatic weeds can compete with the growth of algae in environments where surface waters are P-limited.

CORRELATIONS BETWEEN ALGAL BIOASSAYS AND CHEMICAL ANALYSES TO EVALUATE THE EFFECTS OF WASTEWATER PHOSPHORUS ON RECEIVING WATERS

Nutrient surveys of the Yahara and Rock Rivers found phosphorus in excess of that required by the existing algae and aquatic weeds upstream from the effluents of the cities of Stoughton, Oregon and Madison. The algal bioassays indicated that soluble orthophosphate is the form of phosphorus in river waters that can be used by algae.

Analyses of the phosphorus content of the Madison area lakes - Chemical analyses and bioassays of 58 collections of samples indicate that all the lakes become relatively devoid of P by early summer, but, while Lakes Mendota, Monona, and Waubesa continue to have relatively low P levels until fall overturn, Lake Kegonsa had spectacular increases in its P content during August and September. These were probably associated with septic tank drainage from shoreline cottages. In contrast, during the winter Lake Kegonsa demonstrated a tremendous capacity to assimilate the P added from Lake Waubesa and the smaller tributaries so that relatively little soluble PO_4-P left the lake. These data confirm results obtained in 1943-44.

Sources of phosphorus to the Madison lakes - Comparisons of the availability of lake-water soluble PO_4-P and the soluble PO_4-P of creek or river waters have demonstrated that ground waters and some storm sewer discharges contain P compounds that are not available to algae. The availability of these P compounds appears to increase when the waters flow through widespreads in the river or with certain treatments in the laboratory. Thus, the value of bioassays in conjunction with chemical analyses for evaluating nutrient sources for lakes has been demonstrated.

LAKE RESTORATION EFFECTS AS MEASURED BY THE ALGAL ASSAY PROCEDURE: BOTTLE TEST

Bioassay data, including measurements of algal production, identification of limiting nutrients and the determination of biologically available nutrient concentrations, were obtained for six study lakes over a period of two years. This work was conducted as one element of a more comprehensive data collection program to evaluate lake renewal techniques, particularly aluminum precipitation and hypolimnetic aeration. All assays were conducted on autoclaved samples using the Algal Assay Procedure: Bottle Test.

Algal production in samples from Snake Lake and Horseshoe Lake showed a significant reduction following treatment of the lakes with aluminum. This reduction was most pronounced in hypolimnetic samples. This supports similar findings, based on chemical analyses. Bioassays also showed a distinct shift from nitrogen-limitation to phosphorus-limitation in these lakes.

Two additional lakes, Long and Pickerel, were also treated with aluminum. Bioassay data showed very little change as a result of treatment. Algal production was relatively low in all samples both before and after treatment, and biomass was virtually always limited by phosphorus throughout the study period.

Mirror and Larson Lakes were subjected to an aeration schedule which included periods of total mixing and hypolimnetic aeration. Assay results for Mirror Lake showed an increase in algal production in samples from the epilimnion and a decrease in production in hypolimnetic samples. A shift from nitrogen-limitation to phosphorus-limitation was noted in samples from the hypolimnion. Larson Lake showed a slight increase in algal production in both surface and bottom waters following aeration. A shift in nutrient limitation may have occurred but the effect was less pronounced than that noted in Mirror Lake.

SECTION II

CHEMICAL AND BIOASSAY METHODS FOR NUTRIENT ANALYSES

CHEMICAL ANALYSES OF WATER SAMPLES

Soluble PO_4-P was most frequently analyzed by the stannous chloride method[1] using a 1 cm cell. Analyses of 0.02 mg P/l or more were considered to be significant. Occasional analyses with the ascorbic acid method[1] using 10 cm cells in Beckman DU supported the values obtained with the stannous chloride method and extended the lower limits to at least 0.002 mg P/l.

Total P analyses were by the H_2SO_4-persulfate-autoclave method.[1] The lower limit for these analyses was considered to be 0.02 mg P/l.

TOTAL PHOSPHORUS CONTENT OF ALGAE

Comparisons have been made of two methods of analysis for the total P of three different types of algae containing different amounts of P.

H_2SO_4-HNO_3 digestion - Dried and weighed samples of algae were digested with 1 ml concentrated H_2SO_4 and 5 ml concentrated HNO_3 until clear, neutralized, and analyzed.

H_2SO_4-persulfate digestion - Dried algae plus 1 ml 10N H_2SO_4, 0.4 g potassium persulfate, and 30 ml H_2O were autoclaved for 30 minutes, neutralized, and analyzed. The results of the analyses are presented in Table 1.

It is evident from the results presented in Table 1 that either digestion method can be used for analyses of the total P of algae even when there is nearly a tenfold variation in the total P content of the algae.

Table 1. COMPARISONS OF TOTAL PHOSPHORUS ANALYSES OF ALGAE

			Percentage of phosphorus			
		No. of	H_2SO_4-HNO_3 method		H_2SO_4-persulfate	
Alga	Source	samples	Ave	6[a]	Ave	6[a]
Chlorella pyrenoidosa (Wis 2005)	Gorham's medium	8	1.18	0.091	1.17	0.042
Cladophora sp	Monona Bay 6/25/69	8	0.378	0.016	0.346	0.024
Rhizoclonium sp	L. Mendota 9/7/71	8	0.157	0.008	0.146	0.016

[a] 6 = standard deviation

ANALYSES OF THE TOTAL IRON CONTENT OF SAMPLES

Comparisons have been made of results using two digestion procedures and a direct method for analyzing the iron content of samples. The iron analysis procedure was to: add 1 ml of "FerroZine" iron reagent (Hach Chemical Co. - Note: Caution should be used with this commercial product because it has only a limited shelf-life.) to a 25 ml sample, heat in a boiling water bath, and measure the purple color produced. Iron in the range of 0.1 to 1.6 mg/l could readily be analyzed using 1 cm tubes with this colormetric analysis. The results of the comparison of H_2SO_4-HNO_3 or H_2SO_4-persulfate digested samples with direct analyses of an iron ore and an alga are presented in Table 2.

It is evident that these samples did not require a previous digestion step before analyses using this reagent. The relatively large standard deviation, 6, using the suspension of the iron ore, taconite, is believed to be mainly due to sampling errors due to difficulty in keeping the different-sized particles evenly suspended. Similar tests with *Microcystis*, *Cladophora*, *Pithophora*, and soil extracts also showed that direct analyses of samples were adequate for iron determinations.

ALGAL BIOMASS MEASUREMENTS

The applicability of the different methods of measuring the biomass of algal cultures depends upon the concentration of algae. Relatively dilute cultures can be readily measured using cell counts (haemocytometer or electronic particle counter) or the fluorescence of chlorophyll *a*. More concentrated cultures can be measured using absorbance or dry weight measurements. Ease of use makes fluorometry and absorbance the usual measurements of choice. As a demonstration of the precision within each of these measurements and the factors to be used in converting each to dry weights, a series of replicate (6) tests were carried out using each of the biomass measurements with *Selenastrum* grown in Gorham's medium. The results of these tests are presented in Table 3 as the raw data, means, standard deviations, and 95% confidence limits.

The techniques used for dry weights, centrifuging a known volume and weighing the pellet in tared pans or filtering a known volume through a tared gooch crucible (glass filter pad), are both normally effective measurements. However, the *Selenastrum* culture (Gorham's medium) used for these tests was particularly slimy and caused the gooch filters to plug before reliable

Table 2. COMPARISONS OF IRON ANALYSES

		Iron content					
		Digested samples					
		H_2SO_4-HNO_3		H_2SO_4-persulfate		Direct analysis	
Iron source	No. of samples	Ave	6[a]	Ave	6[a]	Ave	6[a]
Taconite suspension (mg Fe/100 ml)	8	0.368	0.057	0.456	0.152	0.406	0.175
Chlorella pyrenoidosa (Wis 2005) (% Fe)	8	0.070	0.008	0.072	0.005	0.067	0.008

---

[a] Standard deviation

Table 3. STATISTICAL EVALUATION OF THE RESULTS OF THREE TECHNICIANS MAKING BIOMASS MEASUREMENTS OF *SELENASTRUM* BY DIFFERENT METHODS

		Dry weight		Fluorometry		Cell count		Absorbance [b]	
		Centrifuged	Gooch	1% dil	1% filt	Haem $x10^7$/cc	Coulter[a] $x10^5$/cc	750mμ	600mμ
Technician 1		413.3	66.0	45000	23000	3.43	2.83	0.21	0.24
		410.0	0	46000	23000	3.50	2.90	0.21	0.24
		413.3	133.3	47000	23000	3.32	2.92	0.21	0.24
		420.0	100.0	45000	24000	3.18	3.10	0.21	0.24
		416.7	133.3	48000	23000	3.69	3.23	0.21	0.24
		413.3	0	48000	23000	3.11	3.37	0.21	0.24
	Mean	414.4	72.1	46000	23000	3.37	3.06		
	St dev	3.45	61.2	1378	408	0.21	0.21		
Technician 2		420.0	566.7	67000	12000	3.83	3.45	0.20	0.24
		426.7	666.7	65000	18000	3.97	3.54	0.20	0.24
		433.3	666.7	68000	22000	3.41	3.32	0.20	0.24
		420.0	666.7	55000	21000	3.31	3.42	0.20	0.24
		446.7	666.7	59000	14000	3.52	3.27	0.20	0.24
		436.7	633.3	54000	22000	3.47	3.37	0.20	0.24
	Mean	430.6	644.5	61000	18000	3.58	3.40		
	St dev	10.4	40.4	6150	4309	0.257	0.096		
Technician 3		416.7	533.0	58000	26000	3.55	3.59	0.22	0.25
		420.0	200.0	60000	24000	3.49	3.51	0.22	0.25
		433.3	333.3	59000	25000	3.49	3.39	0.22	0.25
		420.0	466.7	60000	26000	3.71	3.76	0.22	0.25
		406.7	366.7	60000	25000	3.62	3.73	0.22	0.25
		350.0	500.0	60000	25000	3.48	3.39	0.22	0.25
	Mean	407.7	400.0	60000	25000	3.56	3.56		
	St dev	29.6	124.7	836.7	752.8	0.092	0.161		
	$\bar{X}$	417.6	287.2	56000	22000	3.50	3.34	0.21	0.24
	St dev $\bar{X}$	11.77	372.2	8386	3606	0.116	0.255	0.01	0.005

[a] Uncalibrated Coulter Counter Model A

[b] Different sample used only for absorbance (105 mg/1)

weights could be filtered. This is a typical result with certain algal cultures, but the technique is usually very effective with planktonic algae collected from the field.

The fluorometry measurements indicate that for relatively dense algal cultures it is best to dilute the sample rather than use a 1% neutral density filter. This is due to the self-shading of dense cultures.

Manual cell counts using haemocytometer slides are the most laborious of all these measurements. However, the presence of contaminating algae or debris in the samples can be detected and evaluated by this method. Also, it can be used with samples containing large amounts of debris that might interfere with some of the other techniques. The use of electronic particle counters makes cell counting less of a chore, but is not applicable to samples with large amounts of debris. The use of an uncalibrated electronic particle counter is justified if one develops conversion factors to convert readings to actual cell counts or dry weights.

The measurement of absorbance (optical density) of algal cultures is one of the easier techniques to be used. It is preferable to use a wave length of light (750 mμ) which does not rely on chlorophyll absorption, since cultures which are deficient in some essential nutrients lose their chlorophyll with increased incubation and, like fluorometry, give low readings for actual final yield measurements. This method should not be selected because of its apparent precision. Absorbance measurements are easy but the apparent precision of the measurements is due to a lack of differentiation between similar cultures. It is a relatively crude measurement.

Table 4. CONVERSION OF BIOMASS MEASUREMENTS OF *SELENASTRUM* GROWN IN GORHAM'S MEDIUM TO DRY WEIGHTS

A concentration of *Selenastrum* of 100 mg/l (dry weight) is equivalent to:

Absorbance (1 cm cells)

750 mμ - 0.20
600 mμ - 0.23

Fluorometry

Turner - 1,300,000 units
Aminco - 3,200 units

Cell counts 8,400,000 cells/ml

The crude conversion table presented in Table 4 can be used to convert one type of biomass measurement to another. From the data in Table 3, it can be seen that these measurements should all be thought of as having an accuracy of only 10%, and one should not place much reliance on less difference in experimental results.

A few precautions should be pointed out when using any of these biomass measurements. It is obvious that, if the size or shape of cells changes during different growth stages or nutrient limitations, measurements of cell counts will not accurately reflect changes in dry weight. It is known that cells of *Selenastrum*, *Chlorella*, and *Microcystis* decrease in relative size as cultures reach old or senescent stages. Therefore, the conversion of cell counts to dry weights must be viewed as a crude convenience. Similarly, the concentration of chlorophyll *a* in algae cultures will increase with increased dry weight, but nutrient limitations and high light intensities will cause the relative amount of chlorophyll *a* to decrease.

Thus, the rates of growth of algal cultures during the early stages of growth can be readily followed and compared by chlorophyll *a* measurements using fluorometry, but measurements of final yields in waters containing different nutrient levels may involve relatively large errors. Finally, increases in absorbance or dry weight with increased age or nutrient supply of algal cultures must be viewed with caution if the texture of the cultures changes(clumping of cells) or inorganic precipitates form. Thus, there are problems involved with any measurement of algal biomass and the conversion from one to another, so it is always important to understand the normal variability to be expected with these measurements and to apply this information in evaluating the significance of results of bioassays.

ANALYSIS OF NUTRIENT CONTENT OF WATER SAMPLES BY GROWTH OF ALGAE

The value of measuring the growth of algae in water samples is that differentiation can be made between total nutrient contents of water samples as obtained by chemical analyses and the nutrients that are available to support the growth of algae under controlled conditions. The concentration of any required nutrient can be determined by measuring the growth attained by selected algal species in dilutions of the water sample or after suitable spikes of other nutrients are added. Standard techniques for this type of bioassay have been presented as the "Algal Assay Procedure" (AAP) by the Environmental Protection Agency.[2]

Three species of algae have been selected for use in growth tests. They are the green alga, *Selenastrum capricornutum* (AAP), and the blue-green algae, *Microcystis aeruginosa* (AAP) and *Anabaena flos aquae* (AAP). All three grow readily in the algal assay medium (NAAM). In order to evaluate the nutrient content of water samples, it is necessary to measure the response of the test alga to different levels of the nutrients in question. Thus, standard curves of the response of the alga to a range of concentrations of a nutrient in NAAM medium are compared to the growth of the alga in the test water. Since the growth of the alga in the test water may be limited by some other nutrients than the one to be measured, the test waters are usually spiked with certain nutrients when measuring the available content of a particular nutrient. In the Midwest, spikes of N, P and Fe have been found to give the most significant results, and combinations of these nutrients are used in measuring nutrient contents of test waters.

In a typical experiment using *Selenastrum* the final yield of this alga would be measured in NAAM medium (to demonstrate that the inoculum used was viable) and in different levels of N, P, and Fe in NAAM medium. The various media would be placed in Erlenmeyer flasks, inoculated so that each culture would have 1,000 cells of *Selenastrum* per ml, and incubated under 400 ft C of light at 24 °C until maximum growth occurred.

The rate of growth of *Selenastrum* in NAAM medium is presented in Figure 1 as the fluorescence attained after different times of incubation. These data indicate that a logarithmic rate of growth occurred between days 1 to 3 or until the growth reached about 1.5 mg/l (150,000 cells/ml); linear growth occurred between days 3 to 7; and, finally, maximal yield (37 mg/l) after 9 days. A general rule would be that, if the culture was visibly green in color, it no longer was in the log-phase of growth. The maximum specific growth rate of *Selenastrum* in NAAM was 2.6/day, and the standard deviation of the data at day 4 (2.54 fluorescent units) was 0.41 (7.6 ± 1.2 mg/l).

The relationship between the growth of *Selenastrum* and the concentration of NO_3-N in NAAM medium is presented in Figure 2 as the dry weight attained after 7 days in cultures containing different concentrations of NO_3-N. It can be seen that in NAAM there is a direct relationship between the growth attained and the concentration of NO_3-N at least up to 2 mg N/l. In more concentrated media, such as Gorham's medium, this direct relationship continues to as high as 20 mg N/l.

As a demonstration of the growth attained by *Selenastrum versus* the concentration of PO_4-P in culture media, comparative tests were carried out in NAAM and in autoclaved Lake Wingra water

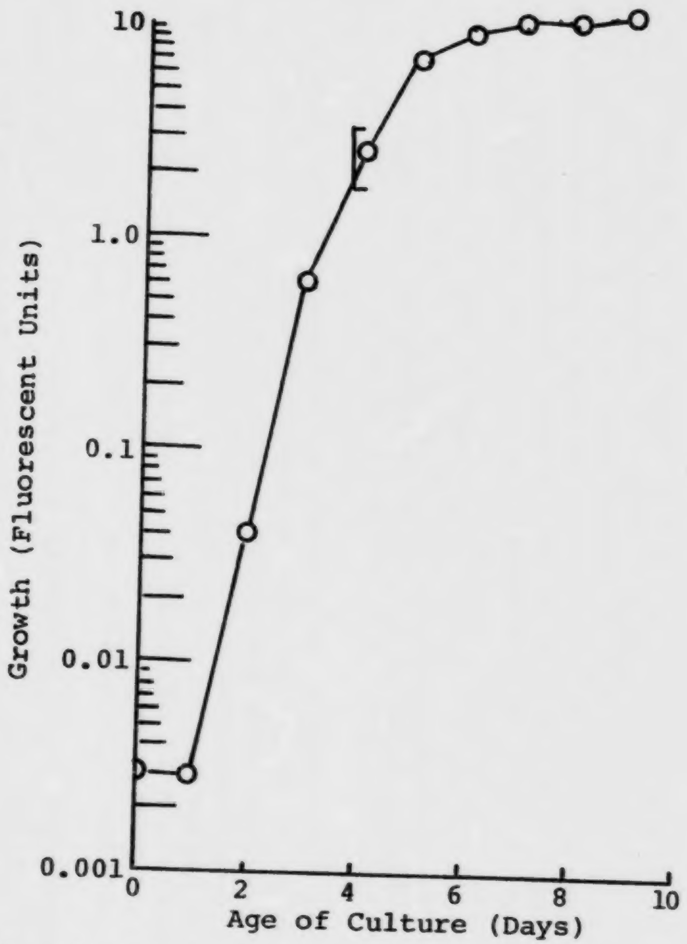

Fig. 1. Rate of Growth of *Selenastrum* in NAAM Medium

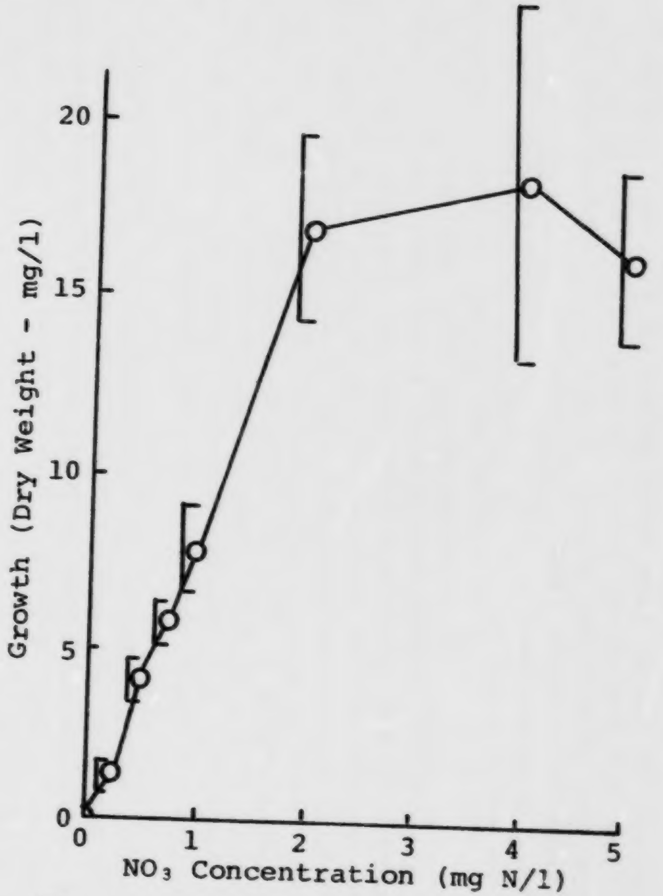

Fig. 2. Growth of *Selenastrum* in Different Concentrations of NO_3-N in NAAM Medium
7 Days Mean and 95% Confidence Limits

(collected 11/20/72) to which were added the NAAM nutrients except PO_4-P. The results of tests with different concentrations of PO_4-P added to these media are presented in Figure 3 as the average growth attained (dry weight) after 7 days of incubation.

It can be seen that the growth of *Selenastrum* in either media was directly related to the PO_4-P concentration at least up to 0.075 mg P/l. Therefore, the response of algae to limiting concentrations of P will be similar whether the medium is a natural water or synthetic culture medium, and standard response curves generated in culture media can be used to predict the available P concentration in media of unknown composition as long as all other nutrients are in sufficient supply.

The growth attained by *Selenastrum* in different concentrations of iron was measured using either NAAM or Gorham's medium containing 1 mg EDTA/l and adding iron as Fe-EDTA. The response to growth-limiting concentrations of iron was similar in either medium, but growth reached higher levels in Gorham's medium than in NAAM when excess iron was present. The relative response to different concentrations of iron was the same whether measured by either fluorometry or absorbance after 7 or 25 days incubation. The results of a typical experiment in Gorham's medium are presented in Figure 4 as the dry weights attained after 25 days incubation.

It is evident that the growth of *Selenastrum* is directly related to the available iron content of the medium up to concentrations near 0.02 mg Fe/l. These results have been confirmed in other media and with *Chlorella, Microcystis,* and *Anabaena.*

The application of algal growth tests to lake waters will provide a measure of the potential nutrient status of the lake water sample, will identify and measure algal growth-limiting nutrients, and will record the biological response to changes in concentrations of algal nutrients. In order to provide such information, the growth of a test alga in different concentrations of N, P, and Fe in control medium is compared with growth attained in a lake water sample with the following additions:

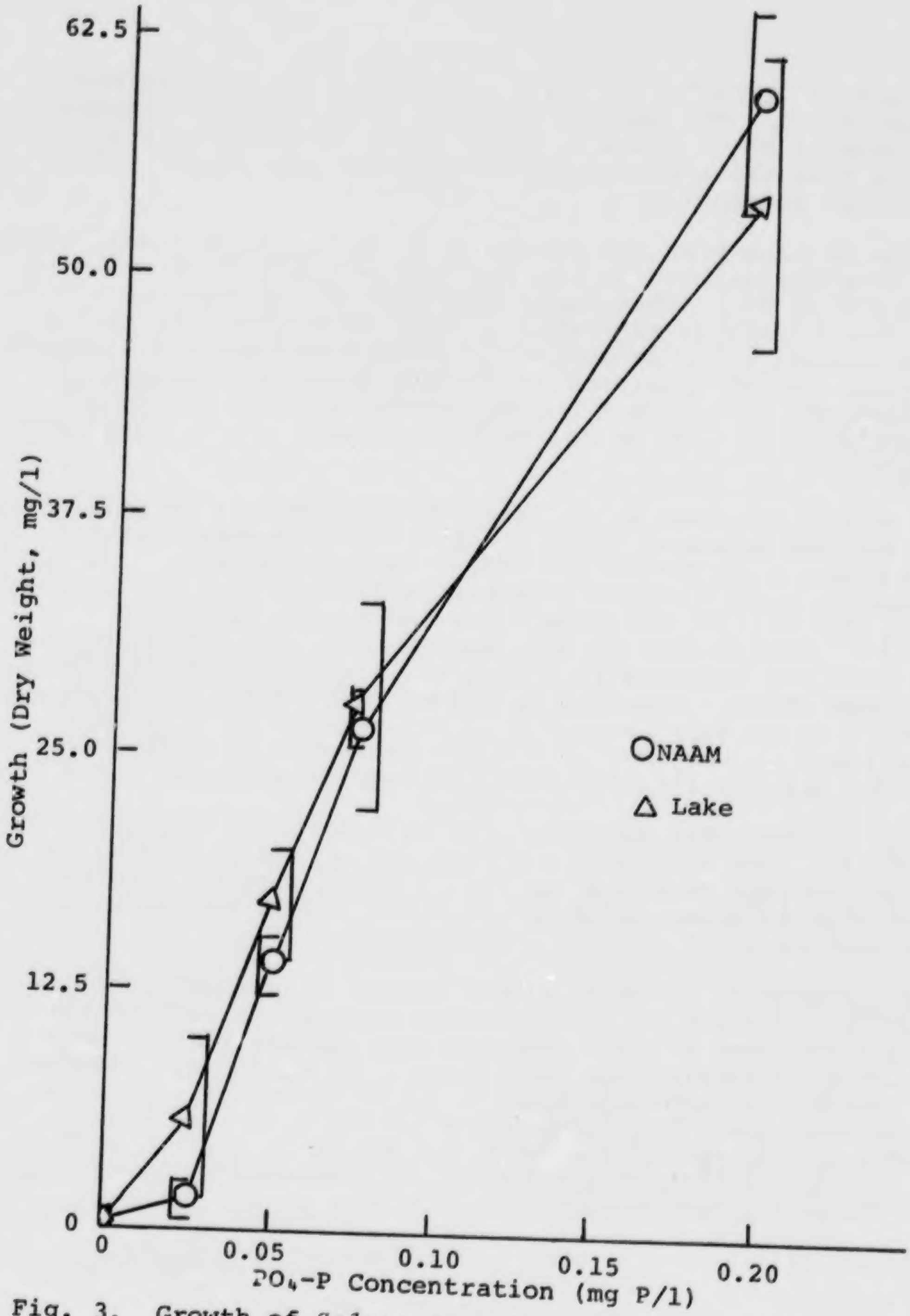

Fig. 3. Growth of *Selenastrum* in Different Concentrations of PO_4-P in NAAM Medium and Lake Wingra Water plus NAAM(-P) Nutrients
7 Days Incubation Mean and 95% Confidence Limits

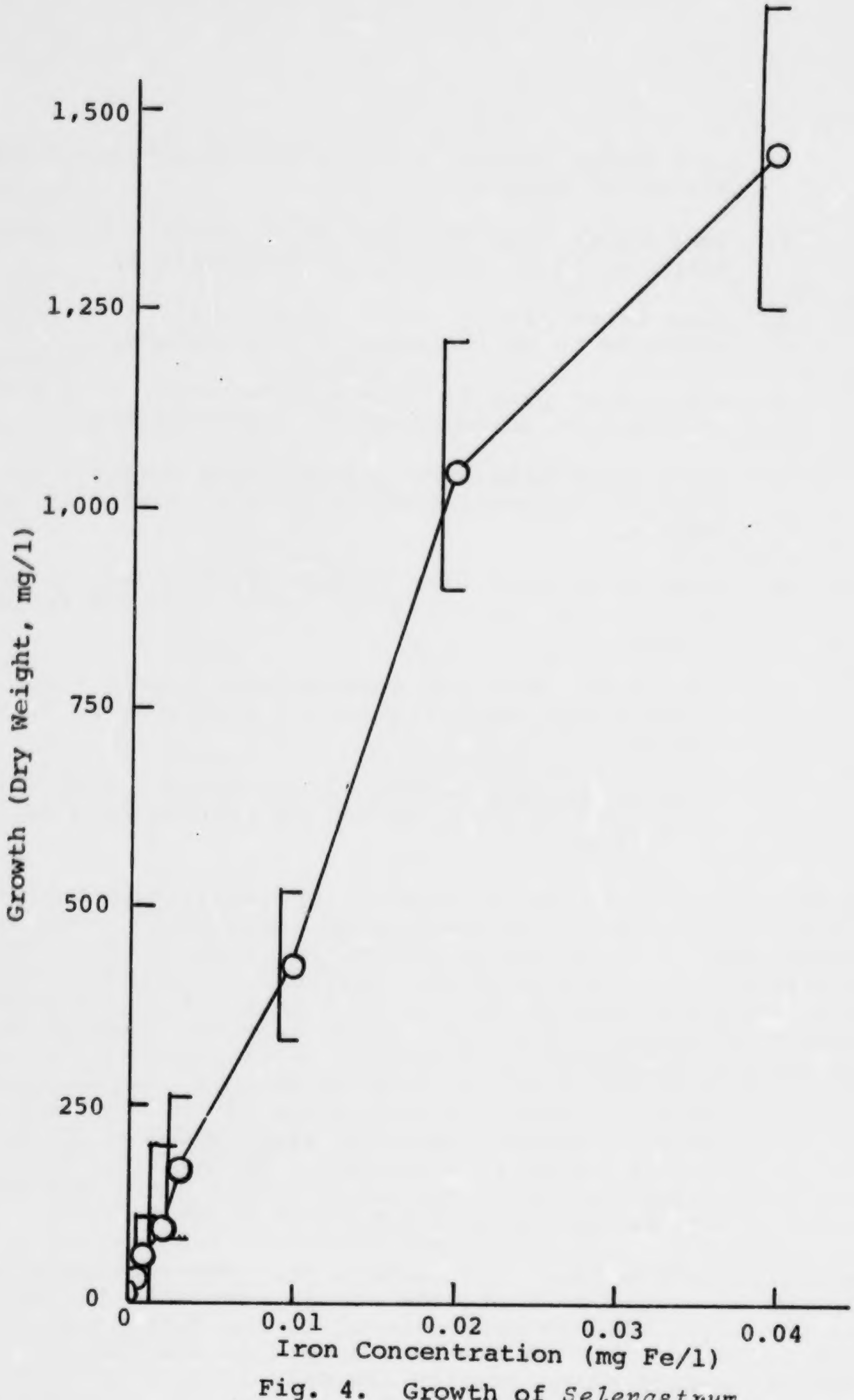

Fig. 4. Growth of *Selenastrum* in Different Concentrations of Iron Gorham's Medium Fe-EDTA 25 Days Mean and 95% Confidence Limits

1. Lake water (as is) - to record relative nutrient status of sample.
2. Lake water plus P spike (NAAM level) - to record response to an increase in available P.
3. Lake water plus N spike (NAAM level) - to record response to an increase in available N.
4. Lake water plus Fe spike (NAAM level) - to record response to an increase in available Fe.
5. Lake water plus N+Fe spikes (NAAM level) - to measure the concentration of available P in the sample.
6. Lake water plus P+Fe spikes (NAAM level) - to measure the concentration of available N in the sample.
7. Lake water plus N+P spikes (NAAM level) - to measure the concentration of available Fe in the sample.
8. Lake water plus N+P+Fe spikes (NAAM level) - to determine if algal growth is limited by a toxic condition.

Lake water samples which support relatively high concentrations of algae will, of course, be more fertile than those which support only sparse growth. If a spike of a single nutrient causes a significant increase in growth it means that this nutrient is the nutrient that is limiting the growth of algae in that lake water sample. Caution should be used in interpreting responses to the addition of NAAM-Fe since this is added in the form of Fe-EDTA, and without further tests it cannot be determined if the response was to Fe or EDTA (a strong chelating agent which might reduce the toxicity of certain chemicals). As has been pointed out, the response of algae to spikes of single nutrients may be limited because two or more nutrients might be in relatively short supply. Therefore, the algae may not respond to an addition of N without also adding Fe. The additions of two spikes at a time will provide enough nutrients so the growth of the algae will be proportional to the concentration of the third nutrient in the lake sample in the case of midwestern waters which usually are only limited by N, P, or Fe contents. If the growth of algae in waters spiked with two nutrients, such as N and Fe at the NAAM level, equals or exceeds the growths attained in the control tests with the highest level of P in NAAM medium, dilutions of the lake water must be tested to obtain the concentration of available P. Lake waters frequently exceed the 0.1 mg P/l concentration usually used in control tests. If algae do not grow in lake waters to which are added N, P, and Fe (or all the NAAM nutrients if other nutrients are suspected to be limiting growth), it must be assumed that some toxic factor is present and special tests will be required to determine the toxic factor.

The relationship between the soluble PO_4-P and available P found with 10 Madison area lake water samples (autoclaved or membrane filtered), which had been fertilized with N and Fe at the NAAM levels in order to make the P of the lake water the only limiting nutrient for the *Selenastrum* used, is illustrated in Figure 5. Growth was measured after 10 days or more, and results with control cultures containing different levels of PO_4-P were used to calculate the available P of the lake water samples. It is evident that for either the autoclaved or membrane-filtered lake waters about 90% of the soluble PO_4-P was available P.

The value of using the growth of algae as a measure of the fertility of water samples is that it demonstrates the level of biologically available nutrients rather than mere total analysis. The responses to spikes of nutrients can be used to predict changes in the eutrophic level of the water with changes in nutrient levels. The main disadvantage is the length of time required for the algae to grow--one week or more.

NUTRITIONAL STATUS OF *IN SITU* ALGAE

The nutritional status of algae which have grown in a particular environment can be used to evaluate whether that environment supplies limiting or surplus nutrients for the growth of algae. In contrast to algal growth tests which measure the biologically available concentration of a particular nutrient in a water sample and require a week or more incubation period, relatively short-term tests can be carried out by measuring changes in certain enzymatic activities or chemical fractions of algae which have been shown to reflect the status of the supply of certain nutrients to the algae at the time sampled.

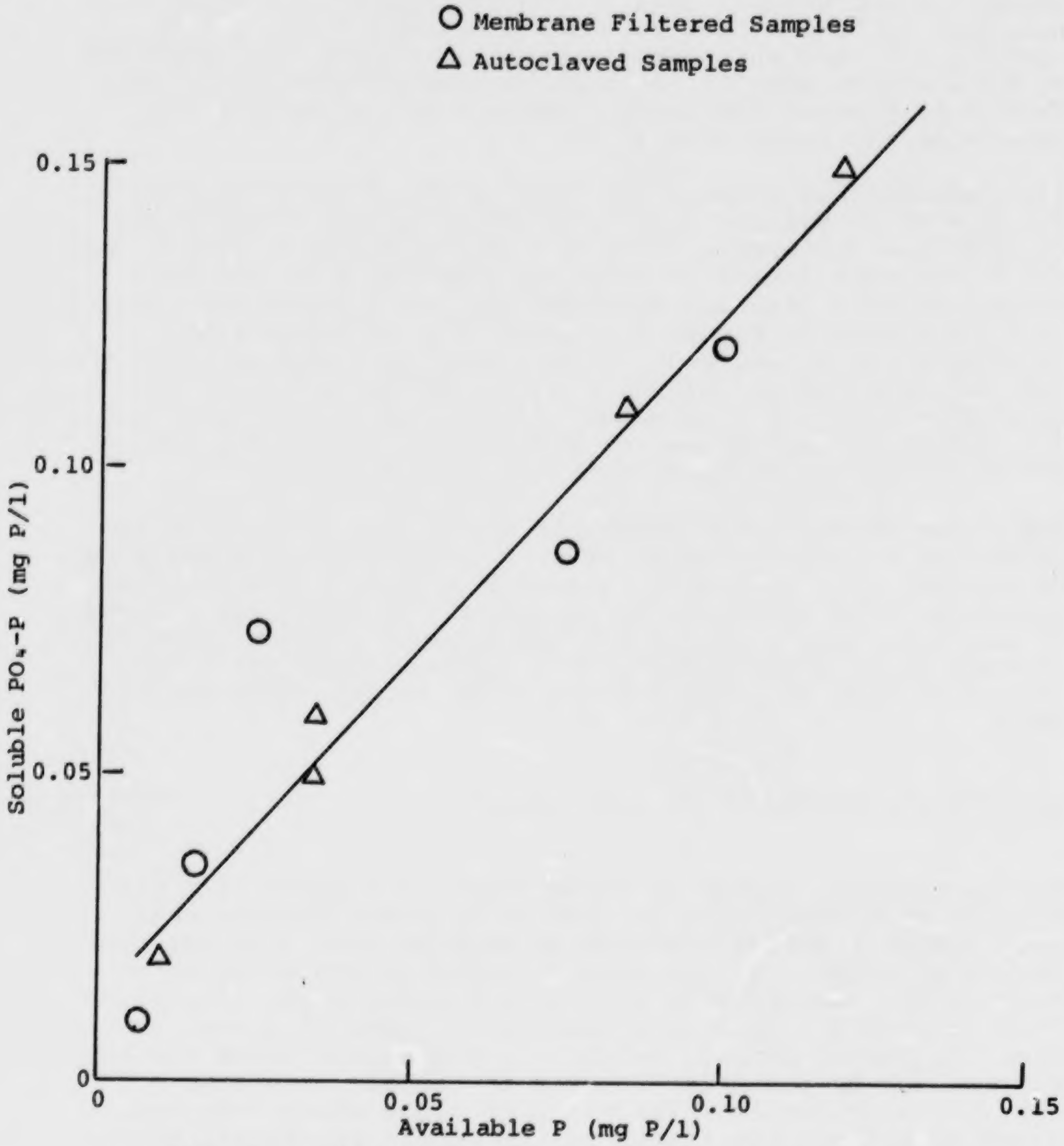

Fig. 5. Relationship between Available Phosphorus (10-Day Growth Test with *Selenastrum*) and Soluble PO$_4$-P in Madison, Wis. Lake Waters Collected 1/4/73

Phosphorus Nutrition of Algae

Several methods are available to differentiate between algae which have surplus P and those that are P-limited. Algae and aquatic weeds containing adequate or surplus P will release more than 0.08 mg PO_4-P/100 mg (dry weight) of plant material when extracted in a boiling water bath for 1 hour.[3] Also, the total P content of plant tissues can be used as a relative measure of the availability of environmental sources of P to the aquatic plants.[4,5] The results of these tests can be confirmed by measuring the alkaline phosphatase activity of the algae or aquatic weeds. It has been found that plants that are P-limited will have as much as 25 times more alkaline phosphatase activity as plants grown with surplus P.

The selection of the method to be used to evaluate the P nutritional status of algae will depend upon the situation. The amount of PO_4-P extracted from algae using a boiling water extraction requires the least amount of equipment. However, the ease with which one can carry out total P analyses using the H_2SO_4-persulfate-autoclave method allows one to readily carry out a large number of analyses by either method. Both methods require a measurement of the dry weight of the algae used in the tests. One usually dries the algae after the extraction procedure, but algae can be dried and then weighed and extracted at a convenient time, much like the procedure used in total P analyses. Comparisons of the results of fresh extracts *versus* extracts of dried algae have been made when algae contained surplus P and when algae were P-limited. The results of representative tests are summarized in Table 5.

The results of these comparisons show that the calculated concentration of extractable PO_4-P from fresh algae is higher than when dried algae are extracted. This is due to the loss of soluble compounds from the fresh algae during the extraction procedure and a resulting lower dry weight measurement. However, the interpretation of the results, whether the algae had surplus or limiting supplies of available P, would only be confused when algae are near the border line between the two conditions. Either sequence of the extraction procedure could be used as long as it is applied systematically to all samples in the series of tests to be compared.

Table 5. COMPARISONS OF THE EXTRACTABLE PO_4-P FROM FRESH *VERSUS* DRIED ALGAE

			Extracted PO_4-P (mg P/100 mg algae)			
			Fresh algae[a]		Dried algae[b]	
Algae	Source	No. of samples	Ave	σ[c]	Ave	σ[c]
Ulothrix sp	Lake Mendota 5/3/72	12	0.67	0.046	0.50	0.037
Spirogyra sp	Lake Kegonsa 5/2/72	8	0.51	0.054	0.35	0.021
Cladophora sp	Lake Mendota 7/16/72	8	0.050	0.013	0.045	0.006
Rhizoclonium sp	Lake Mendota 7/16/72	8	0.067	0.003	0.050	0.008

[a] Algae extracted, dried and weighed
[b] Algae dried, weighed and extracted
[c] Standard deviation

As an example of the results one would get when the extractable PO_4-P content of algae was compared to the total P of algae, tests were carried out with P-limited *Cladophora* sp from the surface of Lake Mendota (7/24/72) and P-surplus *Spirogyra* sp from University Bay Creek (7/24/72), a eutrophic tributary of Lake Mendota. The extractable PO_4-P of the *Cladophora* was 0.071 mg P/100 mg algae (%P) and the total P was 0.16%. The extractable PO_4-P of the *Spirogyra* was 0.42 mg P/100 mg algae and the total P of these algae with surplus P was 0.51%. Thus, either type of analysis could be used to describe the P-nutritional status of *in situ* algae.

As an example of how one can correlate data on the extractable PO_4-P of algae with other environmental parameters, the results of tests at the outlet of Lake Kegonsa are presented in Figure 6. The concentrations of soluble PO_4-P in the water can be compared with the amounts of extractable PO_4-P from *in situ* algae (algae collected at the lake outlet) during April through September, 1972. The species of algae tested were *Spirogyra* sp through mid-May, *Ulothrix* sp until early June, and *Cladophora* sp through the rest of the summer and fall.

It is evident that the extractable PO_4-P from algae sampled at the lake outlet reflected changes in the soluble PO_4-P of the outlet waters. Peaks in either measurement occurred in early May and late summer, whereas relatively low values occurred in mid-May and mid-June. Thus, it can be seen how changes in the extractable PO_4-P of *in situ* algae can be used to evaluate changes in available P in a lake environment. A note of caution: logical responses to changes in environmental conditions can be followed using attached algae (such as *Spirogyra, Ulothrix, Oedogonium,* and *Cladophora*), but planktonic algae do not always reflect environmental changes. Under certain conditions the nitrogen-fixing blue-green algae, *Anabaena, Gloeotrichia,* and *Aphanizomenon,* will be found to be P-limited when the surface waters of lakes are relatively devoid of PO_4-P, but we have never found planktonic non-nitrogen-fixing blue-green algae, such as *Microcystis* or *Coelosphaerium,* to be P-limited under these conditions. The latter algae have been found to be N-limited at certain times, however. Also, one can never be sure whether planktonic algae collected at the surface had not just recently come to the surface and may reflect the nutritional state of subsurface algae.[6]

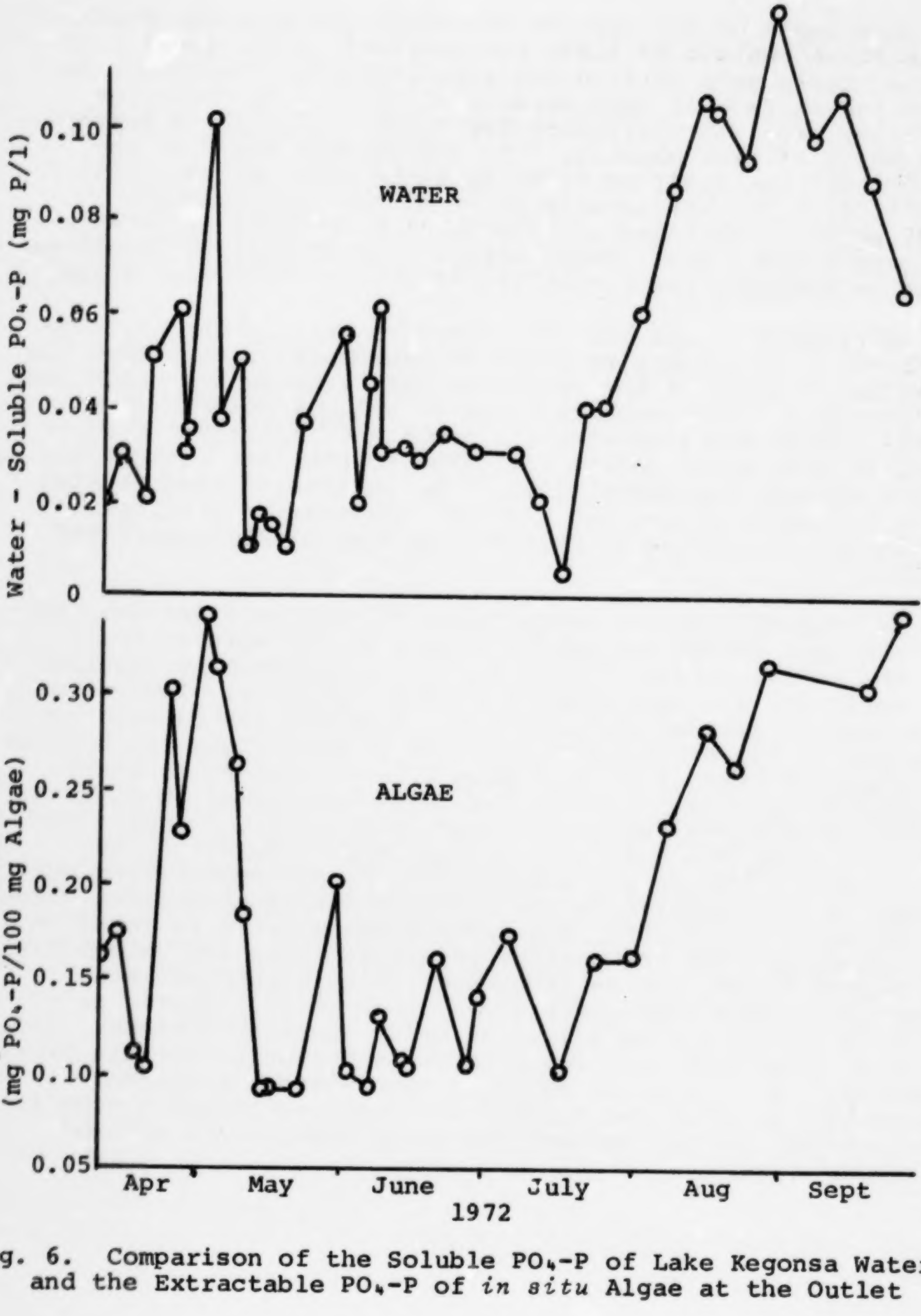

Fig. 6. Comparison of the Soluble PO_4-P of Lake Kegonsa Waters and the Extractable PO_4-P of *in situ* Algae at the Outlet

Nitrogen Nutrition of Algae

Changes in the environmental supply of N to *in situ* algae can be followed by measuring the ammonia absorption rate of the algae in the dark. Algae that are limited by the supply of available N are able to absorb NH_4-N in the dark four to five times more rapidly than plants with adequate N.[7] Thus, one can evaluate the fertilizing effect of rains on N-limited algae. *Cladophora* samples collected from the surface and 0.7-meter depth of Lake Mendota (June 19, 1972) had an average NH_4-N absorption rate of 22 μg N/10 mg algae (dry weight)/hour and had a yellow-green color. After a rain during the night of June 19, the *Cladophora* had an average absorption rate of 6 μg N/10 mg/hour and were bright green. Similarly, a series of rains during mid-July, 1972, caused: the NH_4-N absorption rate of the *Cladophora* of Lake Mendota to decrease from 25 to less than 5 μg N/10 mg/hour, the algae to turn from yellow to green, and, with continued growth in the presence of excess N, the algae became coated with epiphytic diatoms and blue-green algae.[8]

ANALYSIS OF AVAILABLE P BY SORPTION TESTS WITH ALGAE

As an alternative to waiting for algae to grow using absorbed P, one can measure the P absorbed. This can be done by incubating algae in the water sample to be tested and measuring the increase in the P of the algae by either extraction or total P analyses. The amount of P in algae after incubation in known concentrations of PO_4-P is compared with the amount obtained from algae after incubation in the different water samples and is used to calculate the concentrations of available P in the water samples. The algae must be alive because the absorption process is physiological rather than mere adsorption: a mixture of P-limited diatoms from Lake Wingra (12/26/72) was found to readily absorb PO_4-P; the extractable PO_4-P after overnight incubation increased from 0.04% P in control (-P) cultures to 0.30% P in cultures containing 0.1 mg P/l, whereas diatoms that had been killed by two cycles of freezing-thawing had only extractable PO_4-P levels of 0.013 and 0.014% P after overnight incubation in control and 0.1 mg P/l cultures. Field algae to be used do not have to be freshly collected in order to be used to absorb available P from test solutions. Algae (*Cladophora* sp or mixed diatoms) stored in refrigerators (4°C) for as long as 6 months were still capable of absorbing

PO_4-P in quantitative tests. The algae that have been successfully used in sorption tests were usually filamentous types (*Cladophora* (± epiphytes), *Rhizoclonium*, *Odeogonium*, *Ulothrix*, *Microspora*, *Spirogyra*, *Gloeotrichia*, or mixed diatoms) because of the ease with which they could be harvested from sorption solutions. Test solutions were usually harvested by pouring through plankton nets and the algae separated into duplicate samples for analyses. Algae that are relatively P-limited are usually used for sorption tests, but successful tests have been carried out with algae with initial extractable PO_4-P concentrations of as little as 0.02% and as high as 0.14%. The response of two collections of *Cladophora* sp from Lake Wingra (8/16/72 and 9/6/72) to different amounts of PO_4-P added to Lake Wingra water and incubation (10 to 20 mg algae/400 ml) with shaking or aeration for one day is presented in Figure 7 as the average concentrations of extracted PO_4-P from the algae.

It can be seen that there is a direct relationship between the concentration of PO_4-P added to Lake Wingra water (original PO_4-P concentration below 0.02 mg P/l) and the extractable PO_4-P of the *Cladophora* over the range of 0.05 to 0.15 mg P/l. Similar relationships have been found when the total P of the algae was analyzed after incubation with known PO_4-P concentrations, the P content of the algae going from 0.11% P in the medium lacking P to 0.32% P in the medium containing 0.075 mg PO_4-P/l. The data from control cultures in known concentrations of P were used to calculate the available P concentrations in 22 samples of water from 5 lakes in the Madison, Wisconsin area after one-day sorption tests of untreated samples (8/16/72 and 9/6/72). The results of the tests are presented in Figure 8 as the concentrations of soluble PO_4-P chemically analyzed in the waters and the concentrations of available P calculated from the sorption tests.

These data indicate that, for these lake water samples, P analyzed as soluble PO_4-P was available P as measured by the sorption test. A word of caution: certain types of waters (creeks and storm sewers) have been found to have chemicals that analyze as soluble PO_4-P but which are not all available P. As a typical example, the extractable PO_4-P from P-limited *Cladophora* sp from Lake Wingra after 19 hours incubation in lake and creek waters is presented in Table 6 along with the original soluble PO_4-P content and the calculated available P content using results from spiked Lake Wingra water as the controls. It is evident that the soluble PO_4-P of Lakes Waubesa and Kegonsa as well as the added spikes of PO_4-P were 100% available to the *Cladophora*. In contrast,

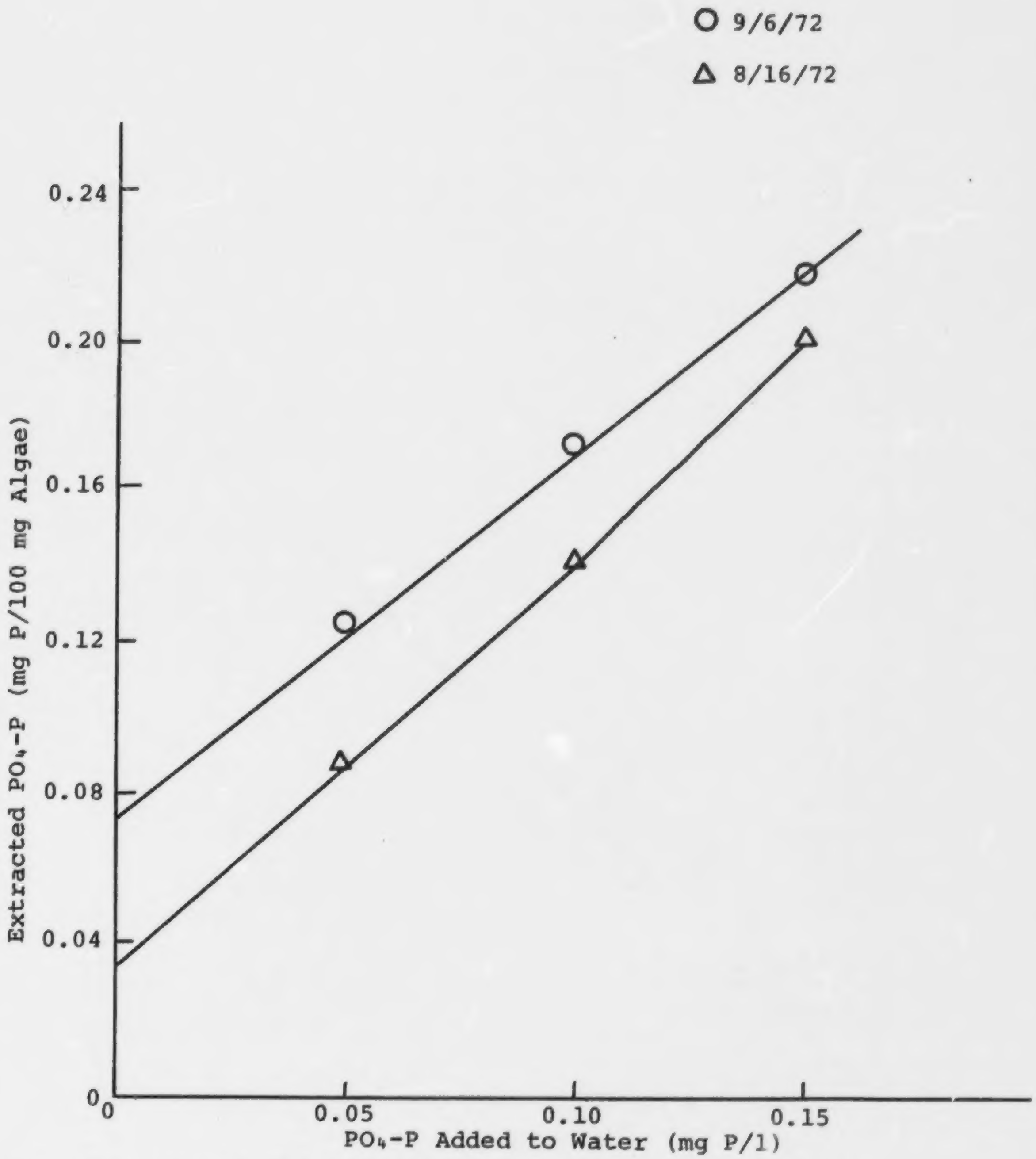

Fig. 7. Concentration of Extracted PO_4-P from Lake Wingra *Cladophora* sp after One-Day Incubation with PO_4-P Added to Lake Wingra Water (10-20 mg algae/400 ml water)

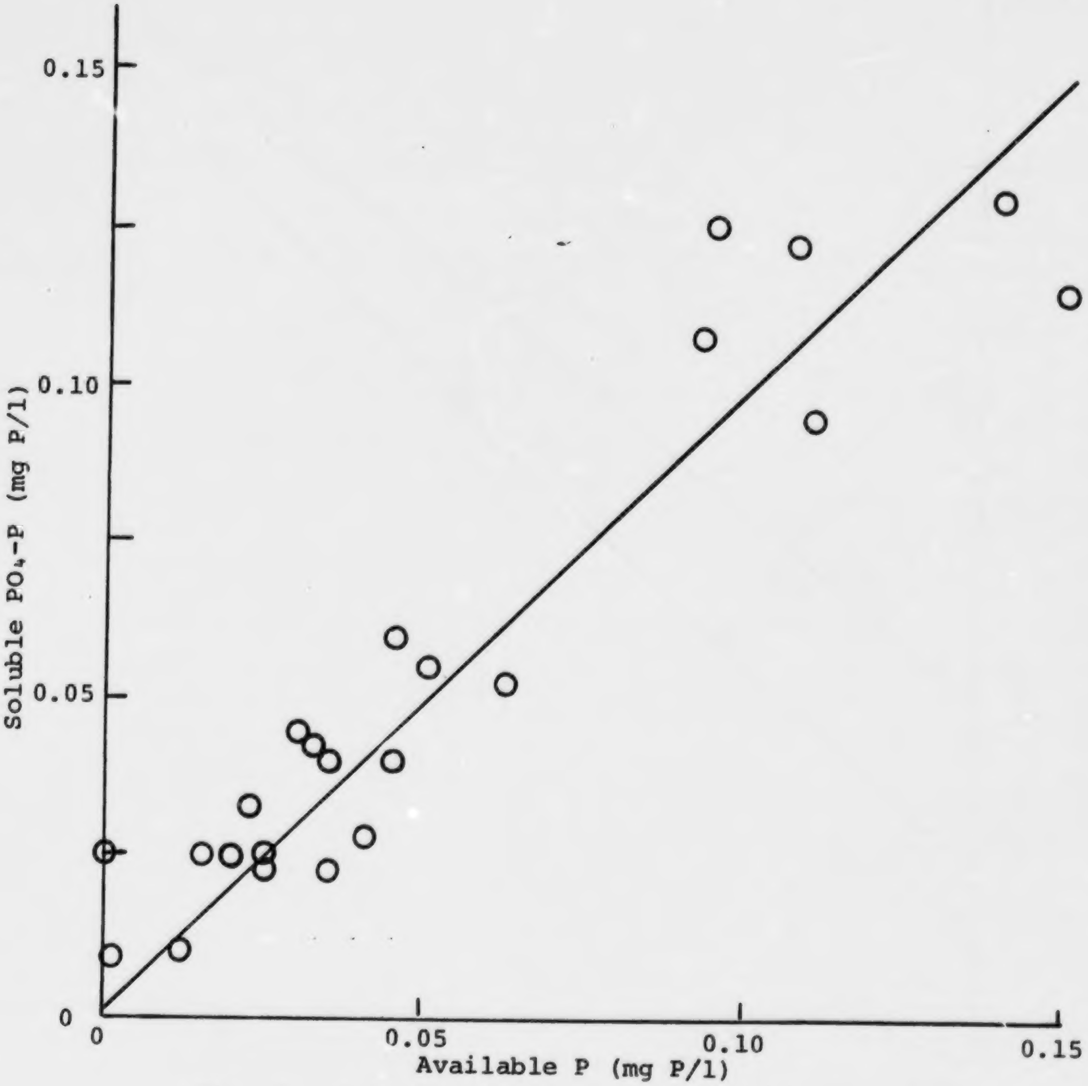

Fig. 8. Relationship Between Available Phosphorus (24-Hour Sorption Tests) and Soluble PO_4-P in Madison, Wisconsin Lake Waters 8/16/72 and 9/6/72 *Cladophora* sp

Table 6. SORPTION OF PHOSPHORUS BY P-LIMITED *CLADOPHORA* SP (LAKE WINGRA) FROM LAKE WATERS *VERSUS* CREEK WATERS

19 hours incubation 15 mg algae/400 ml continuous shaking

Water Sample Source	Added P (mg P/1)	Soluble PO_4-P (mg P/1)	Extracted PO_4-P (mg P/ 100 mg algae)	Calculated available P (mg P/1)	% of soluble P as available P
Lake Wingra	0.0	0.0[a]	0.033	-	-
" "	0.05	0.05	0.062	-	-
" "	0.10	0.10	0.10	-	-
" "	0.20	0.20	0.17	-	-
Lake Waubesa	0.0	0.01	0.047	0.020	100
" "	0.10	0.11	0.11	0.11	100
Lake Kegonsa	0.0	0.074	0.093	0.085	100
" "	0.10	0.17	0.17	0.20	100
Door Creek (at MN)	0.0	0.086	0.059	0.040	46
" " " "	0.10	0.19	0.096	0.090	47
Door Creek (at 12-18)	0.0	0.12	0.062	0.045	38
" " " "	0.10	0.22	0.11	0.11	50

[a] No soluble PO_4-P detectable (less than 0.02 mg P/1).

the P measured as soluble PO_4-P in the creek samples and the spikes of PO_4-P added were only about 50% available. In general, we have found that only 20 to 75% of the soluble PO_4-P of river or creek waters which have not been modified by being below lakes or lake-like widespreads was available P whether measured in short-term sorption tests or growth tests using *Selenastrum*.

The volumes and incubation times used in sorption tests do not appear to be important factors as long as control cultures with different concentrations of PO_4-P are incubated under the same conditions as samples to be tested. As examples, the extractable PO_4-P from samples of algae incubated in volumes from 100 to 1,000 ml containing different amounts of added PO_4-P for 3 to 20 hours are presented in Figure 9. The data show that whether Lake Wingra *Cladophora* is incubated in 100 ml of Gorham's-P medium for 20 hours, or in 1,000 ml of Lake Mendota water (7/14/72) for 4 hours, or whether Lake Mendota *Gloeotrichia* sp (7/31/72) is incubated in 200 ml Gorham's-P medium for 30 hours, there is a direct relationship between the extractable PO_4-P of the algae and the concentration of PO_4-P in the medium tested.

WATER SAMPLE PRESERVATION

The treatment to be given water samples before evaluating their nutrient content will depend upon the test to be used. Usually sorption tests require so little time and space to carry out that tests can readily be carried out on samples as fast as they are received or even in the process of bringing them to the laboratory, if necessary. Since the usual handling procedures in harvesting sorption tests consists of removing the test algae with a forceps or coarse plankton net funnel, most planktonic *in situ* algae in the water sample would not be harvested and analyzed. However, their presence in the sample during the sorption incubation may have an effect on the results because of competition with the test algae. In a previous report (1972) the effects of various planktonic algae (*Gloeotrichia* and mixed plankton) on the P sorbed by *Cladophora* was reported: the extracted PO_4-P of the *Cladophora* incubated alone going from 0.024% P in Lake Wingra water to 0.075% P in lake water plus 0.04 mg PO_4-P/l, whereas in the presence of 17 mg/l of mixed plankton from Lake Wingra the extracted PO_4-P of the *Cladophora* went to only 0.033% P in lake water plus 0.04 mg PO_4-P/l. The majority of *in situ* algae can readily be removed from water samples by plankton nets or centrifugation, and samples can be stored for short periods in the dark or under refrigeration.

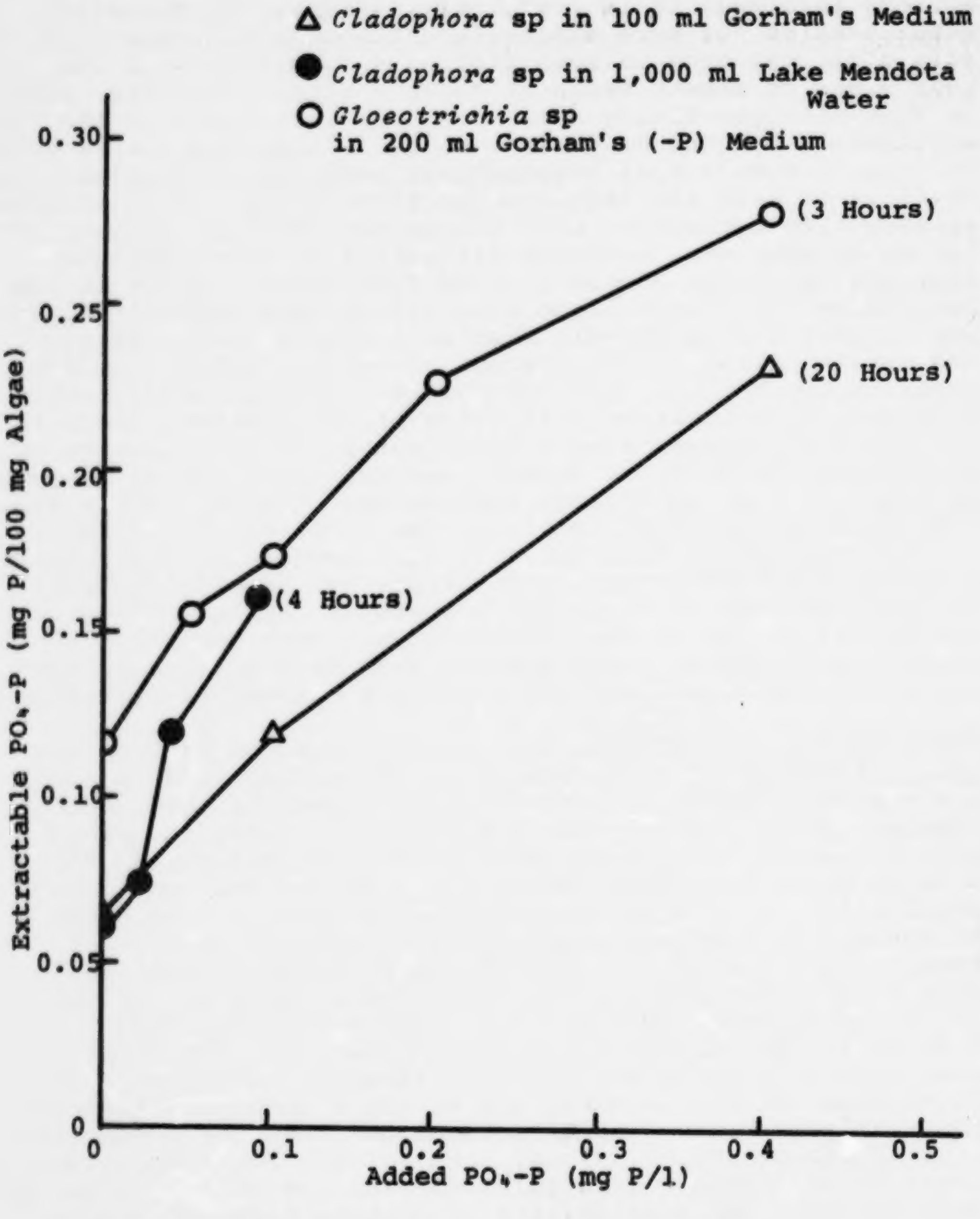

Fig. 9. Sorption of PO_4-P by *Cladophora* sp and *Gloeotrichia* sp in Different Volumes and Different Incubation Times

Since growth tests require a relatively large amount of laboratory space for the number of flasks and long incubations involved, it is frequently necessary to preserve water samples for more appropriate times of analyses. *In situ* algae may grow in some samples and compete with the test algae of growth tests or cause confusing results, such as when nitrogen-fixing algae might grow in tests of the available N content of water samples. Therefore, it is convenient to remove most organisms by membrane filtration (0.45 μ) or kill all organisms by autoclaving. Both of these preservation techniques will affect the nutrient content of the water samples. Membrane filtration removes particles that are insoluble at the time of filtration. Since it has been shown[9,10,11] that algae can utilize some forms of P, N, and Fe that are relatively insoluble (shark teeth, hair, and pyrite crystals), filtration of water samples from four lakes in the Madison, Wisconsin area (January, 1973), which were run after membrane filtration of the samples, indicated all four of the waters were deficient in Fe (stimulation of growth when Fe-EDTA was added), whereas there was no response to added Fe when autoclaved samples were tested. It is known that autoclaving makes available the P, N, and Fe of *in situ* algae, but because the available P of autoclaved and filtered samples of the above-mentioned waters was nearly identical, the availability of Fe in the autoclaved samples was not thought to be due to the killing of the very sparse *in situ* algae. Thus, these water samples must have originally contained adequate available Fe which was removed by filtration.

There are several effects that autoclaving can have on the nutrient status of water samples. The method used to preserve water samples by autoclaving has been to autoclave the samples, cool, gas with CO_2 for 1/4 to 1/2 hour to resolubilize some of the precipitated materials, and aerate for 8 to 12 hours to remove excess CO_2. As has been mentioned, available P, N, and Fe can be released from *in situ* algae in autoclaved samples. During July, 1972, 24 lake water samples were tested for soluble PO_4-P, total P, and available P (sorption tests with *Cladophora*). Only two of the 24 raw or filtered (Whatman #2) samples had more than 0.02 mg P/l as soluble PO_4-P or available P. Six samples that were autoclaved all had significantly higher soluble PO_4-P than the raw samples, and in all 6 autoclaved samples the available P was 2 to 4 times higher than the concentration of soluble PO_4-P. Thus, both soluble PO_4-P and other forms of available P were released from the *in situ* algae by autoclaving. The availability of certain forms of P other than soluble PO_4-P is well documented in this laboratory. One-day sorption tests with P-limited *Cladophora* indicated that, when tested at 0.10 and 0.15 mg P/l, all of the P of pyrophosphate (2.4% soluble ortho PO_4-P) and tripolyphosphate (1.6% soluble ortho PO_4-P) was available, whereas only 0.5% of a concentration of 1.0 mg P/l of phosphate (0.4% soluble ortho PO_4-P) was available. Also, *Cladophora* was able to obtain available P equivalent to at least 0.07 mg P/l from a shark tooth in 1 liter of Lake Mendota water in a 2-day sorption test.

In contrast to the increase of soluble and available P caused by autoclaving lake water samples containing *in situ* algae, autoclaving muddy river water samples caused a decrease of soluble PO_4-P in 11 of 12 samples. This loss in soluble PO_4-P in muddy samples is believed to be due to sorption of P by the mud.[9]

The general conclusions that can be drawn about water sample treatments are:

Raw samples - The nutrient content of untreated stored samples may change because of the growth of algae. Also, *in situ* algae can compete with test organisms in either sorption or growth tests. For sorption tests the effects of *in situ* algae can be removed by reducing their numbers to below competitive status by crude filtration through plankton nets or coarse filter papers. Minimal changes appear to take place in samples stored refrigerated in the dark.

Preserved samples - Membrane filtration will remove competitive organisms to nutrition tests as well as insoluble nutrients which might or might not be available nutrients. Such treatments allow the measurement of nutrients soluble at the time of treatment, that is, what is left in water samples after algae and bacteria are removed. The filtration of samples of more than a few hundred ml, however, requires very special equipment not normal to routine laboratories. Autoclaving causes a release of nutrients from *in situ* algae and an increase in the sorption of nutrients by muds. However, the total available nutrient content of water samples (the availability of nutrients in the water and contributed by the death of *in situ* algae) can be measured after autoclaving. Samples to be stored for long periods could be crudely filtered to remove the majority of *in situ* algae or muds and then autoclaved as a compromise of the effort required and the integrity of results.

REFERENCES

1. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. New York, U.S. Public Health Association, 1971. p. 518-532.

2. Environmental Protection Agency. Algal Assay Procedure: Bottle Test. Washington, D.C., National Eutrophication Research Program, August 1971. 82 p.

3. Fitzgerald, G. P., and T. C. Nelson. Extractive and Enzymatic Analyses for Limiting or Surplus Phosphorus in Algae. J. Phycol. 2:32-37, 1966.

4. Gerloff, G. C. Evaluating Nutrient Supplies for the Growth of Aquatic Plants in Natural Waters. In: Eutrophication: Causes, Consequences, Correctives. Washington, D.C., National Academy of Sciences, 1969. p. 537-555.

5. Fitzgerald, G. P., S. L. Faust, and C. R. Nadler. Correlations to Evaluate Effects of Wastewater Phosphorus on Receiving Waters. Water and Sewage Works 120:48-55, January 1973.

6. Fitzgerald, G. P., and G. F. Lee. Use of Tests for Limiting or Surplus Nutrients to Evaluate Sources of Nitrogen and Phosphorus for Algae and Aquatic Weeds. Madison, Water Chemistry Program Report, University of Wisconsin-Madison, 1970. 31 p.

7. Fitzgerald, G. P. Detection of Limiting or Surplus Nitrogen in Algae and Aquatic Weeds. J. Phycol. 4:121-126, 1968.

8. Fitzgerald, G. P. Some Factors in the Competition or Antagonism among Bacteria, Algae and Aquatic Weeds. J. Phycol. 5:351-359, 1969.

9. Fitzgerald, G. P. Evaluations of the Availability of Sources of Nitrogen and Phosphorus for Algae. J. Phycol. 6:239-247, 1970.

10. Fitzgerald, G. P. Aerobic Lake Muds for the Removal of Phosphorus from Lake Muds. Limnol. & Oceanog. 15:550-555, 1970.

11. Fitzgerald, G. P. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters, Allen, H. E., and J. R. Kramer (ed.). New York, John Wiley and Sons, 1972. p. 147-170.

SECTION III

RATES OF PHOSPHORUS UTILIZATION BY ALGAE AND AQUATIC WEEDS

Introduction

The need for information as to the relative rates of phosphorus utilization by algae and aquatic weeds became apparent when it was found that certain algae become P-limited at times during the summer.[1,2,3] It was important to know how algae and aquatic weeds would compete for P when ecosystems are managed to limit the quantities of biologically available P. Since it has been shown that once algae or aquatic weeds have absorbed P, it becomes unavailable to other plants until the original plant is dead (live algae and aquatic weeds do not excrete significant quantities of nutrients), the rate at which different species of plants sorb P from solution is as important as the relative amounts of P available to algae and aquatic weeds.[1] Therefore, the rate of growth of different species of plants is not as important as their rate of P sorption if the quantity of available P is going to be used to control the biological productivity of aquatic ecosystems.

The amount of growth attained by a plant with P absorbed from a culture medium is the ultimate answer to the quantity of available P in a culture medium. In order to utilize the relatively rapid sorption process in conjunction with the long incubation required for growth, algae or aquatic weeds can be exposed to sources of P and then transferred to P-free medium for growth. This process is also advantageous when comparing the availability of different forms of a nutrient, some of which may degrade to other forms during long incubations. Results of this type of test have been presented in studies with the green alga, *Selenastrum capricornutum* (PAAP),[1] and the duckweed, *Lemna minor*,[4] in which it was shown that *Selenastrum* could sorb maximal quantities of ortho- and pyrophosphate in less than 2 hours whereas 4 to 6 hours were required by duckweed to sorb significant quantities of P from these two sources. Although the results of these tests are based on the growth attained by the organisms, which is the ultimate answer for most comparisons, the 2- to 3-week incubations required cause one to seek comparative methods involving less time.

Other methods that can be used to measure the rate of sorption of P by algae or aquatic weeds without waiting for the plants to grow with the sorbed P are measurements of P lost from the culture medium or analysis of the plants for P sorbed. Chamberlain and Shapiro[5] used the loss of P from algae culture media to compare the biologically available P of lake waters with different chemical analyses for P. The present study uses the rate of loss of P from different media to compare the rates of sorption by algae and aquatic weeds. In addition, the comparative ability of algae to sorb P is measured by increases in the extractable PO_4-P[6] or total P of the algae. The main emphasis has been placed on whether different species of aquatic plants could sorb P to the exclusion of other species or whether the plants tested merely share available P. The ecological implications of these results and other studies will be discussed from the point of what might take place in the balance of algae *versus* aquatic weeds in lakes which become P-limited due to the control of P sources to the lake environment.

Materials and Methods

The algae and aquatic weeds used for most of these studies were field collections from the Madison, Wisconsin area. Comparative tests indicated that the rates of P sorption measured were not influenced when different lake waters were compared with Gorham's (-P) medium. Therefore, many of the results reported are for lake waters with naturally high P concentrations (sample from hypolimnion of Lake Mendota) or lake waters supplemented with P to bring the level up to that sometimes found to be present in hypolimnion samples (0.4 mg P/l).

The rate of decrease of soluble PO_4-P from media caused by algae or weeds was followed by the molybdate-stannous chloride method.[7] Approximately 100 mg (dry weight) of plants were placed in 2 liters of media containing 0.4 mg PO_4-P/l. Duplicate 50 ml samples were removed at different times for analyses of the soluble PO_4-P remaining. At the end of the experiment the actual dry weight of the plant material was measured so results could be reported as mg P sorbed/100 mg algae or weeds/hour. In this manner, many replicates of several different algae could be tested simultaneously under similar environmental conditions.

The amount of P sorbed by algae was measured by placing duplicate 5 mg samples of algae in 500 or 1,000 ml of water containing different levels of P (0.02 to 0.1 mg P/l), incubating the cultures overnight, and then analyzing the algae for sorbed P and dry weight. The sorbed P was either analyzed as the extractable PO_4-P or as the total P of the algae. In either case, results with control cultures containing no P were used in comparison with known concentrations of P. The ease with which filamentous algae, such as *Cladophora*, can be separated from planktonic species by the use of a coarse screen makes it easy to compare the P sorbed by different types of algae when in mixed cultures.

The analysis of extractable PO_4-P of algae has been described by Fitzgerald and Nelson.[6] The total P analysis procedure was that of Gales et al.[8] In well equipped laboratories either method is readily carried out with about the same expenditure of time, but the analysis of extractable PO_4-P requires less equipment and technical effort. When using duplicate 5 mg samples of algae per 500 or 1,000 ml, either type of analysis for sorbed P could detect differences caused by 0.02 mg P/l in the culture medium after 18-24 hours incubation. The volumes used in comparative tests are not important in rate studies since the effects of 0.01 or 0.02 mg PO_4-P added to 10 mg of algae can be detected whether the algae are incubated in 100 or 1,000 mg. In addition, sorption tests with Lake Wingra *Cladophora* sp indicate that the same rates of sorption take place in 400 ft candles of light as in the dark.

Results and Discussion

Over 50 tests have been carried out measuring the rates of P sorption by algae or aquatic weeds based on the loss of soluble PO_4-P from the media. In a typical experiment, *Cladophora* sp collected from Lake Mendota and found to contain adequate P (0.18 mg extractable PO_4-P/100 mg algae) were compared with P-limited *Cladophora* sp (0.029 mg PO_4-P/100 mg) from Lake Wingra. Approximately 170 mg of either algae were placed in 2,000 ml of Lake Mendota water which was supplemented with 0.4 mg PO_4-P/l. Analyses of the PO_4-P of the lake water over a 4-hour period are summarized in Figure 10.

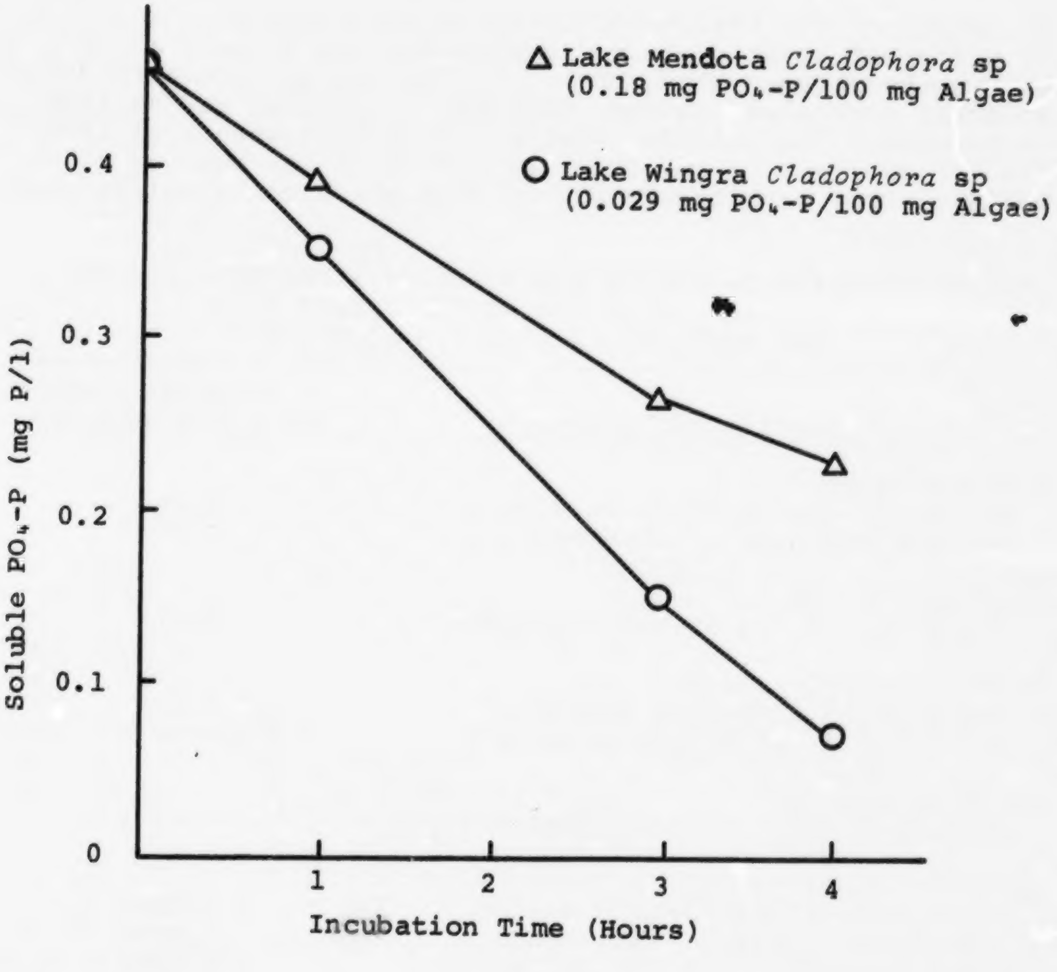

Fig. 10. Rates of Phosphorus Sorption by *Cladophora:* P-Limited *versus* Surplus P

170 mg Algae/2,000 ml Lake Mendota Water

It is evident that the P-limited algae from Lake Wingra were able to remove P from the medium at a more rapid rate than algae collected from Lake Mendota. The rates of P sorbed during this experiment were 0.057 and 0.035 mg PO_4-P/100 mg algae/hour, respectively. Later in the summer, other samples of *Cladophora* sp from Lake Mendota were found to be P-limited (less than 0.08 mg PO_4-P extracted/100 mg algae) and had P sorption rates equal to those measured with *Cladophora* sp from Lake Wingra.

A summary of the results of tests of the rates of P sorption by different aquatic plants is presented in Table 7 as the mg P sorbed/100 mg algae/hour. The plants are identified as to their source and whether they were P-limited at the time of testing. The test for whether the plants were P-limited was the analysis of the PO_4-P extracted in one hour by a boiling water bath treatment. Plants which contain less than

Table 7. RATES OF PHOSPHORUS SORPTION BY AQUATIC PLANTS
BY ANALYSIS OF THE MEDIUM *VERSUS* TIME
100 mg/2,000 ml plus 0.4 mg PO_4-P/1

Plant material and source	Sorption rate (mg P/100 mg/hour)
Cladophora sp	
P-limited (0.05% P[a])-Lake Wingra	0.05
Surplus-P (0.15% P)-Lake Mendota	0.02
Rhizoclonium sp	
P-limited (0.07% P)-Lake Mendota	0.07
Pithophora oedogonium	
Low-P (0.1% P)-P-free medium	0.03
Surplus-P (0.4% P)-lab aquaria	none
Gloeotrichia sp	
Surplus-P (0.3% P)-Lake Mendota	0.05
Myriophyllum sp	
Plant tips (0.2% P)-Lake Mendota	0.008
Tip leaves (0.2% P)-Lake Mendota	0.010
Basal leaves (0.04% P)-Lake Mendota	0.015
Lemna minor (duckweed)	
P-limited - P-free medium	0.003

[a] % P means mg PO_4-P extracted/100 mg algae

0.08 mg PO_4-P/100 mg algae are considered to be P-limited.[6] The general conclusions that can be drawn are that the rates of P sorption by algae are fairly similar, and the rates by aquatic plants are generally slower under the conditions of these tests.

It is evident that the rates of P sorption by the different aquatic plants are affected by whether or not the plants are P-limited. The most conspicuous effect of surplus P conditions was with the green alga, *Pithophora oedogonium*, which was cultured in aquaria to prevent the growth of other algae.[9] Growth under these conditions of surplus P (0.4 mg extractable PO_4-P/100 mg) caused the algae to contain a maximal amount of P and, under the conditions of the test, they did not sorb additional P. When these algae were cultured in P-free medium for a few weeks, the extractable PO_4-P was reduced to 0.1 mg P/ 100 mg and the algae sorbed P at a rate similar to *Cladophora* sp under similar conditions. Similarly, the P sorption by basal leaves of *Myriophyllum* sp from either Lake Mendota or Wingra was always more rapid than by leaves from the tips of the plants. The P content of tip leaves is higher because under P-limiting conditions P migrates in the plant to the more actively growing portions of the plant.

The significance of the rates of P sorption by algae in the range of 0.02 to 0.07 mg P/100 mg algae/hour is evident when the chemical composition of algae is considered. For instance, field collections of *Cladophora* sp contain 0.1 to 0.4 mg P/ 100 mg algae, depending upon whether or not they are P-limited at the time of collection. The measured rates of P sorption indicate that the time required to double the P content of P-limited *Cladophora* would be about 2 hours. This corresponds to the 2 hours required for the maximal sorption of P by *Selenastrum* in the exposure and subculture for growth tests reported earlier.[1]

Measurements of P sorption based on decreases in the concentrations of soluble PO_4-P in culture media are readily carried out, but are not direct measurements of the P sorbed by the plants. In addition, no direct evidence can be obtained by this method of the rate of P sorption by individual species of algae in mixtures. For the latter type of information, increases in the P content of the algae or aquatic weeds rather than mere loss from solution must be measured. The methods for measuring the increase in sorbed P that we have used were analysis of either the extractable PO_4-P or the total P of the algae. Since the sorption rate of P by the blue-green alga, *Gloeotrichia* sp from Lake Mendota, was known to be equal to or greater than that of field collections of *Cladophora* sp, measurements were made of the amount of P that each of these algae could sorb when limited or surplus quantities of PO_4-P were added to mixtures of the two algae. In the first test approximately 12 mg samples of algae alone or together were placed in 1,000 ml of Lake Mendota water containing either no measurable P, or with the addition of 0.04, 0.08, or 0.12 mg PO_4-P. The *Cladophora* and *Gloeotrichia* were recovered from the culture media by using a large-mesh screen for the *Cladophora* and a fine plankton net for the *Gloeotrichia*. The extractable PO_4-P of the algae after 20 hours incubation alone or together in the different media was measured and is summarized in Table 8.

Table 8. COMPARATIVE RATES OF PHOSPHORUS SORPTION BY *CLADOPHORA* SP AND *GLOEOTRICHIA* SP FROM LAKE MENDOTA WATER
12 mg algae/1,000 ml Lake Mendota water

	Extractable PO_4-P (mg P/100 mg algae) after 20 hours incubation			
		Lake water		
Algae tested	Lake water	+0.04 mg PO_4-P/l	+0.08 mg PO_4-P/l	+0.12 mg PO_4-P/l
Cladophora-alone	0.08	0.19	-	-
Gloeotrichia-alone	0.40	0.55	-	-
Cladophora in mixture	0.09	0.13	0.18	0.18
Gloeotrichia in mixture	0.40	0.43	0.66	0.66

It can be seen that the *Cladophora* used were relatively P-limited (low extractable PO_4-P values after incubation in unsupplemented lake water) when compared to the *Gloeotrichia*. When tested alone in the presence of 0.04 mg PO_4-P either alga was able to sorb P such that their extractable PO_4-P increased considerably. When the algae were incubated together with 0.04 mg PO_4-P/l neither alga prevented the sorption of P by the other alga, and the result was that both had only limited increases in their extractable PO_4-P. In the presence of 0.08 mg PO_4-P/l both algae were able to sorb what appeared to be maximal quantities of P since no further increases in extractable PO_4-P occurred in the presence of 0.12 mg PO_4-P/l.

Thus, it would appear that when equal quantities of *Cladophora* and *Gloeotrichia* are mixed they merely share any available P.

In other experiments, the effect of different amounts of *Gloeotrichia* on the sorption of P by *Cladophora* was measured. A summary is presented in Table 9 as the average amounts of extractable PO_4-P of duplicate 5 mg samples of *Cladophora* sp incubated alone or with 9, 18, or 36 mg of *Gloeotrichia* sp in Lake Mendota waters for 24 hours.

Table 9. EFFECT OF COMPETITION BY *GLOEOTRICHIA* SP
ON THE SORPTION OF PHOSPHORUS BY *CLADOPHORA* SP
10 mg *Cladophora*/1,000 ml Lake Mendota water
24 hours incubation

Quantity of *Gloeotrichia* (mg/l)	Extractable PO_4-P of *Cladophora* sp ($mg\ PO_4$-P/100 mg algae)		
	Lake water	Lake water + 0.04 mg P/l	Lake water + 0.06 mg P/l
none	0.07	0.20	-
9	0.06	0.19	0.21
18	0.06	0.13	0.17
36	0.06	0.14	0.19

It is apparent that the presence of the *Gloeotrichia* did not affect the extractable PO_4-P of the *Cladophora* in unsupplemented Lake Mendota water. When 0.04 mg PO_4-P/l was added to the lake water, the higher quantities of *Gloeotrichia* prevented the *Cladophora* from sorbing as much P as when incubated alone. However, with an increase to 0.06 mg P/l, the *Cladophora* was able to sorb nearly maximal amounts of P even in the presence of more than four times as much *Gloeotrichia*.

Further tests of the competition of algae for P were carried out with the plankton and *Cladophora* sp from Lake Wingra. There is usually a heavy bloom of plankton in Lake Wingra, and it usually consists of a large number of different species characterized by their small colonial forms. In a typical test, the effect of live and killed plankton (17 mg/l) on the

P sorption by *Cladophora* during 15 hours incubation in Lake Wingra waters was tested. A summary of the results as the averages of duplicate samples is presented in Table 10.

Table 10. THE EFFECT OF LAKE WINGRA PLANKTON ON THE SORPTION OF PHOSPHORUS BY *CLADOPHORA* SP
15 to 20 mg Lake Wingra *Cladophora*/1,000 ml Lake Wingra water
24 hours incubation

	Extractable PO_4-P of *Cladophora* after incubation (mg PO_4-P/100 mg algae)	
Plankton (17 mg/l)	Lake water	Lake water + 0.04 mg PO_4-P/l
None (centrifuged)	0.024	0.075
Live (as is)	0.026	0.033
Dead (autoclaved)	0.080	0.13

The presence of live plankton did not affect the P sorption by the *Cladophora* in unsupplemented Lake Wingra water, but caused less P to be sorbed by the *Cladophora* in the lake water with an additional 0.04 mg PO_4-P/l. When the plankton was killed by autoclaving the lake water, releasing available P for sorption by the *Cladophora*, the extractable PO_4-P increased from 0.024 to 0.080 mg P/100 mg algae. This dead plankton had no effect on the further sorption of P by the *Cladophora*. When 0.04 mg PO_4-P was added to the autoclaved lake water, the extractable PO_4-P of *Cladophora* increased as much as that of *Cladophora* from supplemented centrifuged lake water. Therefore, the sorption of P by mixtures of algae results in competition between species for the available P, but there is no evidence that one type of algae can prevent another type from sorbing P.

The general results of these experiments have shown that different algae or aquatic weeds have P sorption rates which would indicate that they probably would compete with each other for supplies of available P, but that no plant that was P-limited could be prevented from using a portion of the available P. This would mean that in a lake environment the quantity of available P in the lake water would not preferentially influence the growth of different species of algae or aquatic weeds. Studies with algae and the rooted aquatic weed, *Lemna minor* (duckweed), have indicated that neither type of plant can utilize the P of lake muds under aerobic conditions.[4,10] However, duckweed was able to utilize P from lake muds that were stratified so some depths reached by the roots of the duckweed became anaerobic and PO_4-P was released to the interstitial water of the muds. Thus, in an aquatic environment in which the sources of P to the surface waters are controlled so the waters are stripped of available P during the growing season of the algae and aquatic weeds, there will probably be preferential conditions for the growth of aquatic weeds through the use of P in layers of lake muds which is unavailable to algae. From a management point of view, the growth of aquatic weeds is preferable since they can be more readily harvested from selected areas than can growths of planktonic algae.

References

1. Fitzgerald, G. P. Evaluations of the Availability of Sources of Nitrogen and Phosphorus for Algae. J. Phycol. 6:239-247, 1970.

2. Fitzgerald, G. P., and G. F. Lee. Use of Tests for Limiting or Surplus Nutrients to Evaluate Sources of Nitrogen and Phosphorus for Algae and Aquatic Weeds. Madison, Water Chemistry Program Report, University of Wisconsin-Madison, 1970. 31 p.

3. Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burris. Acetylene Reduction Assay for Determination of Phosphorus Availability in Wisconsin Lakes. Proc. National Acad. Sci. 66:1104-1111, 1970.

4. Fitzgerald, G. P. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters, Allen, H. E., and J. R. Kramer (ed.). New York, John Wiley and Sons, 1972. p. 147-170.

5. Chamberlain, W., and J. Shapiro. On the Biological Significance of Phosphate Analysis; Comparison of Standard and New Methods with a Bioassay. Limnol. and Oceanogr. 14:921-927, 1969.

6. Fitzgerald, G. P., and T. C. Nelson. Extractive and Enzymatic Analyses for Limiting or Surplus Phosphorus in Algae. J. Phycol. 2:32-37, 1966.

7. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. New York, U.S. Public Health Association, 1971. p. 518-532.

8. Gales, M. E., Jr., E. C. Julien, and R. C. Kroner. Method for Quantitative Determination of Total Phosphorus in Water. Amer. Water Works Assn. 58:1363-1368, 1966.

9. Fitzgerald, G. P. Some Factors in the Competition or Antagonism among Bacteria, Algae and Aquatic Weeds. J. Phycol. 5:351-359, 1969.

10. Fitzgerald, G. P. Aerobic Lake Muds for the Removal of Phosphorus from Lake Waters. Limnol. and Oceanogr. 15:550-555, 1970.

SECTION IV

THE RELEASE, SORPTION, AND AVAILABILITY TO ALGAE OF PHOSPHORUS FROM LAKE MUDS

Introduction

The availability to algae of the P in lake muds has been questioned because we recently found phosphorus-limited algae growing in intimate contact with lake muds containing phosphorus. It also has been shown by Swingle *et al*[1] that the muds of eutrophic fish ponds do not release enough available P to support the desired level of phytoplankton, but that fertilization with only P would maintain the productivity of phytoplankton and harvestable fish crops. Our studies with the duckweed, *Lemna minor*, have also indicated that this rooted aquatic weed could not obtain available N or P from aerobic lake muds.[2] In contrast to these results with attached algae, phytoplankton, and duckweed, Martin *et al*[3] have shown that the aquatic weed, *Najas* sp, obtained the inorganic nutrients required for growth from lake muds through a relatively weak root system. Our duckweed studies have also indicated that if the roots of this plant could penetrate mud layers that have become anaerobic, the plant could obtain P for growth from the mud. It appears, therefore, that one must be careful in discussing the availability of nutrients from lake muds because, while algae and aquatic weeds appear to be alike in not being able to compete with the forces holding P to aerobic lake muds, aquatic weeds which can send roots into layers of anaerobic lake muds which contain PO_4-P in the interstitial waters can utilize source of P that are not normally available to algae.

Layers of lake muds that are anaerobic contain PO_4-P[4] which should be available for the growth of algae. Therefore, it was of interest to study under what conditions available P was released from muds to surface waters. Both *in situ* and laboratory studies were made of the release of PO_4-P from disturbed lake muds. These results were compared with analyses during a dredging operation. The phosphorus status of *in situ* algae during the operation and after lake storms has been correlated to laboratory studies of the availability of the P of lake muds. Proof of use of a nutrient is best provided by growth responses of the test alga. Such tests require relatively long incubations. Shorter tests have been developed that rely on the use of enzymatic responses or changes in the chemical composition of algae. All three types of responses have been used in evaluating lake or river muds as sources of P for algae. In addition, a discussion is presented of the advantage rooted aquatic weeds will have over algae in eutrophic aquatic environments which have been stripped of their soluble P.

Results and Discussion

Release of phosphorus from lake muds -

It has been pointed out that *in situ* lake muds are anaerobic and contain PO_4-P in the interstitial waters.[4] A series of experiments were carried out following the release of PO_4-P from lake muds disturbed as a simulation of the feeding of fish or of wave action. A stainless steel column, 30 cm in diameter and 75 cm long, was embedded vertically in a lake bottom such that the top extended over the water surface. The mud at the bottom of the column was mixed by hand to a depth of 10 cm. The soluble PO_4-P in the water column was measured before and after mixing to follow the release of P. Tests were carried out in three lakes: Lake Wingra, 45 cm depth, grey sandy silt; Monona Bay (Lake Monona), 50 cm depth, grey sandy silt; University Bay (Lake Mendota), 45 cm depth, black muck. The results of soluble PO_4-P analyses are summarized in Table 11 as the averages of duplicates.

It is evident that soluble PO_4-P was released to the overlying waters from all three lake muds, the amounts of P released ranging from 0.007 mg P/l in Lake Wingra to about 0.04 mg P/l in Lake Mendota. However, in all cases the soluble PO_4-P contents of the enclosed water decreased with time after initially high levels. When the muds were remixed after

Table 11. RELEASE OF PHOSPHORUS
FROM DISTURBED *IN SITU* LAKE MUDS
Muds stirred to 10 cm depth in 35 liter column.

	Soluble PO_4-P in lake waters (mg P/l)		
Time	Lake Wingra	Monona Bay	University Bay
Pre-mix	0.011	0.009	0.025
0 - Time	0.018	0.042	0.063
5 Minutes	0.018	0.016	0.049
10 Minutes	0.014	0.012	0.037
15 Minutes	0.012	0.012	0.043
15 Minutes + Remix	0.020	0.021	0.053

15 minutes, there was another peak in PO_4-P from the anaerobic interstitial waters and the sorption of the P by the muds under the aerobic conditions in the overlying waters.

Laboratory studies of this initial release of P were carried out by carefully filling a 300 ml jar with mud, sealing the jar, and later mixing with 3,000 ml of lake water from the sampling sites. It was found that muds from Monona Bay released about 0.04 mg P/l within 5 minutes and muds from University Bay released about 0.05 mg P/l. The sorption of PO_4-P by these muds was followed by aerating the 3,000 ml mud-water mixtures to establish aerobic conditions, adding 0.6 mg PO_4-P, and following the concentration of PO_4-P remaining in solution with time. The results with muds from Monona Bay and University Bay are summarized in Table 12 as the percentage of PO_4-P remaining in solution at different times.

These data indicate that these muds which released PO_4-P when under anaerobic conditions were able to sorb added P when tested under aerobic conditions. It is therefore assumed that the decrease in soluble PO_4-P of *in situ* mixtures of muds and aerobic waters was due to this ability of muds to sorb P when changed from anaerobic to aerobic conditions. Therefore, in natural situations, P could be released to the overlying waters by disturbing anaerobic mud layers, but aquatic plants would have to compete with aerobic muds for the P.

Table 12. SORPTION OF PHOSPHORUS BY AEROBIC LAKE MUDS
300 ml mud/3,000 ml lake water + 0.6 mg PO_4-P

Time (minutes)	Percentage soluble PO_4-P remaining	
	Monona Bay	University Bay
1	74	61
5	44	58
15	28	52
20	18	30

The release of PO_4-P from lake muds by a dredging operation has also been studied. Lake muds were being dredged from Lake Monona to a marsh near Murphy's Creek (outlet of Lake Wingra to Lake Monona). During a period of dredging, it was found that the supernatant waters which entered Murphy's Creek contained 0.13 mg soluble PO_4-P/l and 0.64 mg total P/l whereas the waters upstream contained less than 0.02 mg PO_4-P/l. Since downstream samples of Murphy's Creek contained considerably less soluble PO_4-P than the supernatant entering the creek, tests were made of the sorption of PO_4-P by mud in the dredging supernatant. The soluble PO_4-P concentration of aerated dredging supernatant which contained fine grey sand to silt decreased to 0.076 mg P/l after one day and 0.030 mg P/l after two days. When dredged mud was suspended in river water supplemented with 0.4 mg PO_4-P/l, 20, 30, and 45% of the PO_4-P of 75 ml of the water was sorbed in 10 minutes by 120, 240, and 480 mg mud, respectively. Thus, it appears that muds disturbed by this dredging operation released soluble PO_4-P to Murphy's Creek, but that the sorption of P by aerobic muds took place.

Availability of phosphorus to *in situ* algae -

Since the previous studies had indicated that P could be released from disturbed lake muds, it was of interest to determine how important such sources of P were to *in situ* algae. Samples of *Cladophora* sp were collected from 3 sites in Lake Wingra and 2 sites in Monona Bay on days when wind storms had caused the surface muds to be suspended and to actually coat the algae at the time of sampling. In addition, samples were collected of *Spirogyra* sp which had grown through layers of mud (0.09% P) settled from the dredging operation in Murphy's Creek. The total P of the muddy water from which the algae were collected and the extractable PO_4-P of the algae were measured and are summarized in Table 13 as averages of triplicates.

Table 13. COMPARISONS OF THE TOTAL PHOSPHORUS CONTENT OF MUDDY WATERS WITH THE EXTRACTABLE PO_4-P OF *IN SITU* ALGAE

Sample	Source	Alga	Total P of water (mg P/l)	Extracted PO_4-P (mg P/100 mg algae)
1	Wind-stirred Lake Wingra	*Cladophora* sp	0.30	0.027
2	Wind-stirred Lake Wingra	*Cladophora* sp	0.31	0.015
3	Wind-stirred Lake Wingra	*Cladophora* sp	0.82	0.023
4	Wind-stirred Monona Bay	*Cladophora* sp	0.19	0.039
5	Wind-stirred Monona Bay	*Cladophora* sp	0.19	0.072
6	Dredged muds, Murphy's Creek	*Spirogyra* sp	0.28	0.049
7	Dredged muds, Murphy's Creek	*Spirogyra* sp	0.26	0.059

There was considerable total P in the wind-stirred waters surrounding the *Cladophora* samples, ranging from 0.2 to 0.8 mg P/l, but the extractable PO_4-P of these algae indicated they were still P-limited (algae containing more than 0.08 mg extractable PO_4-P/100 mg are considered to have adequate available P).[5] The extractable PO_4-P of samples of *Cladophora* from Lake Wingra before and after this wind storm was about 0.02 mg P/100 mg algae. *Spirogyra* sp and *Zygnema* sp upstream from the dredging area of Murphy's Creek contained 0.03 to 0.07 mg extractable PO_4-P/100 mg algae. It is therefore evident that although there was considerable P stirred up by the wind storm and the *Spirogyra* had been covered by the dredged muds, these *in situ* algae had not been able to absorb significant amounts of P from the different environments.

Laboratory bioassays with lake muds -

Since the ultimate test of the availability of a source of P is whether or not a plant can use it for growth, the availability of the P of the muds from the dredging operation was tested using the growth of the green alga, *Selenastrum capricornutum* (AAP). The alga was inoculated (50,000 cells/ml) into Gorham's (-P) medium to which were added different levels of PO_4-P or dredged mud from Murphy's Creek. The growth of *Selenastrum* after 12 days incubation in aerated cultures is summarized as absorbance measurements in Table 14.

Table 14. THE AVAILABILITY OF PHOSPHORUS FROM DREDGED MUDS FOR *SELENASTRUM CAPRICORNUTUM* (AAP)
50,000 cells/ml Gorham's (-P) medium 12 days incubation

Phosphorus source	P-concentration (mg P/l)	Growth attained (Absorbance, 1 cm, 750 mμ)
none	0.0	0.015
PO_4-P	0.025	0.030
PO_4-P	0.05	0.045
PO_4-P	0.075	0.070
PO_4-P	0.10	0.090
mud	0.08	0.005
mud	0.20	0.005
mud	0.50	0.005
mud + PO_4-P	0.20 + 0.20	0.090

It is evident that the growth of this alga was dependent upon the PO_4-P added to the culture medium, twice as much *Selenastrum* being produced with 0.02 mg PO_4-P/l as in P-free medium.

The P in the form of dredged mud was not available for the growth of *Selenastrum* under these aerobic conditions, a concentration of 0.5 mg lake mud P/l giving less growth than 0.025 mg PO_4-P/l. When PO_4-P (0.2 mg P/l) was added to a culture with 0.2 mg mud P/l, the *Selenastrum* was able to grow on the added PO_4-P, showing that the lack of growth with other mud samples was not due to the mud being toxic to the alga.

The availability of the P of dredged lake muds was also tested with P-limited N_2-fixing blue-green algae. It has been shown that the rate of fixation of N_2 by certain blue-green algae is dependent upon whether the algae have adequate P. The exposure of P-limited algae to available PO_4-P for as little as 15 minutes will allow the algae to absorb enough P so that increased capability of the algae to fix N_2 can be measured by the acetylene-reduction method. In a typical test, 12 mg samples of *Anabaena flos aquae* (Ind 1444), which had been cultured under limiting P conditions, were added to 225 ml of Gorham's (-P) medium to which was added PO_4-P or mud from the Murphy's Creek dredging operation. After 2 hours incubation in the light, the algae were centrifuged and the ethylene produced by triplicate 4 mg samples of the *Anabaena* in 1 ml volumes was measured after 30 minutes exposure to acetylene in the light. The results as the average n moles of the ethylene produced/mg algae/30 minutes are summarized in Table 15.

Table 15. THE AVAILABILITY OF PO_4-P AND DREDGED MUD PHOSPHORUS TO PHOSPHORUS-LIMITED *ANABAENA FLOS AQUAE* (IND 1444) AS MEASURED BY THE REDUCTION OF ACETYLENE TO ETHYLENE
2 hours incubation Gorham's (-P) medium

Phosphorus source	Concentration (mg P/l)	Ethylene produced (n M/mg algae/30 min)
none	0.0	1.6
PO_4-P	0.005	3.3
PO_4-P	0.01	5.4
PO_4-P	0.02	10.8
dredged mud	0.14	1.6
dredged mud + PO_4-P	0.14 + 0.02	4.2

The fact that the *Anabaena* responded to increased levels of PO_4-P by increased ethylene production indicates that it was originally P-limited and was able to utilize the added PO_4-P, twice as much activity being measured in the presence of 0.005 mg P/l as in P-free medium. The *Anabaena* was not able to utilize the 0.14 mg P/l in the form of mud under these conditions, but because the PO_4-P added to the mud was utilized, the mud could not contain a toxic factor. Other tests carried out in Murphy's Creek water to which PO_4-P or lake muds were added also indicated that P-limited *Anabaena* could not utilize lake mud P.

The bioassays carried out thus far have been with aerobic suspensions of lake muds which contained no measurable soluble PO_4-P. In order to determine if there was available P in muddy waters which contained some soluble PO_4-P as well as a greater amount of total P, tests were carried out with a river water and the sorption of P by P-limited *Cladophora*. The chemical composition of *Cladophora* after exposure to different levels of P can be used as an indication that the algae were able to sorb P under the conditions of the test. Previous studies have indicated that the extractable PO_4-P and extractable total P of *Cladophora* could be used to measure the available P in culture media.[6] In the present test, *Cladophora* sp from Lake Wingra was incubated in 500 ml of Lake Mendota water which was supplemented with different levels of PO_4-P. After incubation overnight, the algae were divided into duplicate samples and the extractable PO_4-P or total P of the algae measured. The results are summarized in Table 16 as the average P concentrations of the algae.

The concentration of extractable PO_4-P or of the total P of the samples of *Cladophora* sp from two locations were measured after incubation in PO_4-P supplemented Lake Mendota water and 20 or 40% dilutions of muddy Crawfish River water in Lake Mendota water. The samples of known concentrations of PO_4-P in Lake Mendota water were to be control cultures which would serve to determine the concentrations of available P in the dilutions of Crawfish River water. In one series, two 5 mg samples of a bright green *Cladophora* collected from a shaded area of Murphy's Creek were incubated in 1,000 ml of supplemented Lake Mendota water. In the other series, two 10 mg samples of pale yellow *Cladophora* from near the surface of Lake Wingra were incubated in 500 ml of supplemented Lake Mendota water. The average total P concentrations of duplicate samples of the algae after 24 hours incubation are summarized in Figure 11.

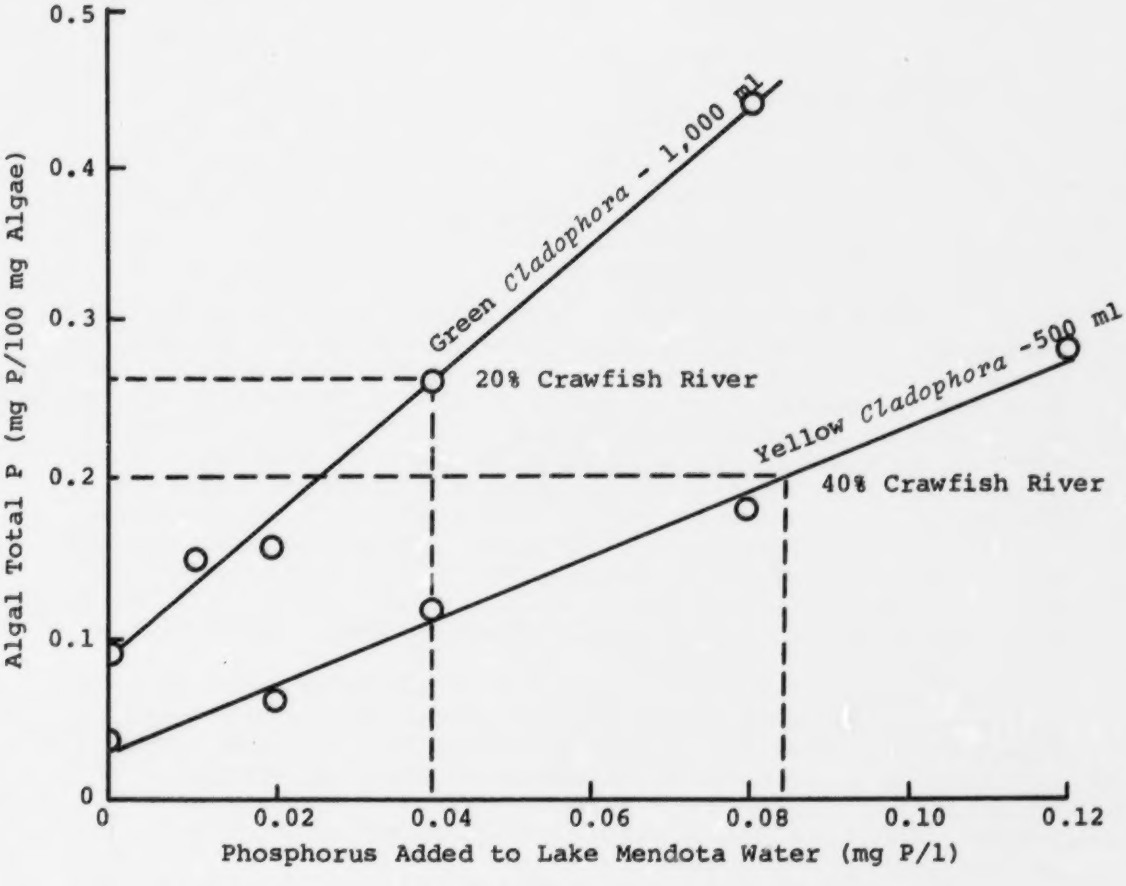

Fig. 11. Total Phosphorus Content of *Cladophora* sp Incubated with Different Phosphorus Levels

24 Hours Incubation

Table 16. CHANGES IN COMPOSITION OF LAKE WINGRA
CLADOPHORA SP AFTER INCUBATION
WITH DIFFERENT CONCENTRATIONS OF PHOSPHORUS
10 mg *Cladophora*/500 ml Lake Mendota water
20 hours sorption

Phosphorus concentration added to Lake Mendota water (mg PO_4-P/l)	*Cladophora* phosphorus concentration (mg P/100 mg algae) Extractable PO_4-P	Total P
0.0	0.076	0.085
0.02	0.092	0.13
0.04	0.15	0.17
0.08	0.22	0.30

It is evident that the total P of either type of *Cladophora* increased when the algae were incubated 24 hours in the presence of increasing levels of PO_4-P in Lake Mendota water. From the concentrations of total P of the *Cladophora* in the dilutions of Crawfish River water in Lake Mendota water, it can be calculated that 0.20 to 0.21 mg/l of available P was present in the Crawfish River sample. Chemical analyses of the river water showed that it contained 0.26 mg soluble PO_4-P/l and 0.61 mg total P/l, thus indicating that most of the soluble PO_4-P was available to the *Cladophora*, but there was a lot of total P present in the muddy water that the algae were not able to utilize under these aerobic conditions.

Similar tests of the availability of the P of aerobic lake muds, using as a measurement the growth of the duckweed, *Lemna minor*, have also indicated that the P of lake muds was not available even when the roots of this aquatic weed were in intimate contact with aerobic muds for as long as 8 weeks. However, this is not a natural environment for aquatic weeds. As has been pointed out, lake muds are layered so that they contain areas which are anaerobic with PO_4-P in the interstitial waters. The advantage of duckweed over algae as a bioassay organism is that the availability of the *in situ* P of layered lake muds can be measured. The roots of aquatic weeds can penetrate an anaerobic environment while the rest of the plant remains in an aerobic environment, such as occurs in normal growth in a lake. Consequently, an experiment was carried out comparing the availability of the P of lake muds to duckweeds floating so far above the muds that they could only absorb P which passed through an aerobic column of medium with duckweeds whose roots could penetrate the mud and presumably extend into any anaerobic environment developed in the mud. Two fronds were placed in 20 cm culture tubes containing Gorham's (-P) medium. To four tubes containing 25 ml of Lake Wingra mud (2.0 mg total P/tube), 30 ml of Gorham's (-P) medium were added so the duckweed floated 8 cm above the surface of the mud. The second set of mud tubes contained only 5 ml of Gorham's (-P) medium so the duckweed floated only about 1 cm from the mud surface. The results of the experiment as the average number of fronds per test condition after 6 weeks incubation are graphically summarized (next section, Figure 12).

Little growth (5 fronds) of the duckweed took place in the P-free Gorham's medium, but 27 fronds developed in the presence of 0.005 mg PO_4-P. It is evident that no available P was released to the overlying medium in the case where the mud containing 2 mg of total P was overlain by 30 ml of Gorham's (-P) medium since the duckweed in these tubes had no more growth than those with only Gorham's (-P) medium. There was considerable growth (27 fronds) of the duckweed whose roots penetrated the mud layers which were presumed to have developed an anaerobic environment during the long incubation period (6 weeks). Observations of these cultures for an additional 8 weeks also indicated that even with additional incubation the duckweed in cultures in which the roots could not touch the mud layers did not grow any farther.

These tests thus indicate how tests can be carried out to contrast the availability of P in lake muds to algae or floating aquatic weeds *versus* that to aquatic weeds whose roots can penetrate to anaerobic layers of mud. As one would expect from chemical studies of the release of PO_4-P to the overlying aerobic waters of lakes, there is available P in lake muds that are under anaerobic conditions. One should be aware, however, of the fact that PO_4-P released from anaerobic lake muds into an aerobic environment can be competitively sorbed by muds under the aerobic environment.[6]

References

1. Swingle, H. S., B. C. Gooch, and H. R. Rabanal. Phosphate Fertilization of Ponds. In: Proc. 7th Annual Conference, S.E. Assoc. Game and Fish Comm. Hot Springs, Oct. 1963. p. 213-218.

2. Fitzgerald, G. P. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters, Allen, H. E., and J. R. Kramer (ed.). New York, John Wiley and Sons, 1972. p. 147-170.

3. Martin, J. P., Jr., B. N. Bradford, and H. G. Kennedy. Factors Affecting the Growth of *Najas* sp in Pickwick Reservoir. Muscle Shoals, National Fertilizer Development Center, TVA. F70-ACD2. 1970. 47 p.

4. Lee, G. F. Eutrophication. Eutrophication Information Program, University of Wisconsin, Madison, Wis. Occasional Paper No. 2. Sept. 1970. 39 p.

5. Fitzgerald, G. P., and T. C. Nelson. Extractive and Enzymatic Analyses for Limiting or Surplus Phosphorus in Algae. J. Phycol. 2:32-37, 1966.

6. Fitzgerald, G. P. Aerobic Lake Muds for the Removal of Phosphorus from Lake Muds. Limnol. and Oceanogr. 15:550-555, 1970.

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SECTION V

DUCKWEED (*LEMNA MINOR*) FOR NUTRITIONAL BIOASSAYS

Introduction

The aquatic plant duckweed (*Lemna minor*) is an example of a rooted aquatic plant that is readily cultured under laboratory conditions. Its characteristic flotation makes it readily harvested or transferred from medium to medium, and simple frond counts can be used as an approximate measure of its growth (increase in dry weight) under different environmental conditions.

Materials and Methods

The duckweed used in these tests has been cultured in this laboratory under algae-free conditions for several years. Treatments of field collections of duckweed frond with chlorine had made it free of other plants. It was maintained in Gorham's medium, but most nutritional tests were carried out using supplemented Gorham's medium containing 20 mg Ca/l (double normal concentration), EDTA (7 mg/l), and minor elements of ASM medium. Preliminary tests indicated that duckweed transferred from Gorham's medium to medium lacking some essential nutrient would increase from an original inoculum of 2 fronds per 50 ml to 10 or more fronds, whereas fronds from Gorham's medium containing growth-limiting amounts of the nutrient of interest exhibited little subsequent growth when transferred to media lacking that essential nutrient. Consequently, the inoculum (usually 2 fronds/50 ml) for most tests came from plants transferred from the supplemented Gorham's medium to media lacking N, P, or Fe and were incubated in the latter media for a week or two. When transferred to fresh media lacking the nutrient of interest, these fronds usually only increased from the original inoculum of 2 fronds to 4 or 5 fronds. The length of incubation required to detect differences between the numbers of fronds in different levels of an essential nutrient was about 2 weeks and maximal growth required 3 to 4 weeks incubation.

Results and Discussion

Most data accumulated using duckweed in nutritional bioassays were reported as the number of fronds produced in the cultures. Early experiments were recorded as both frond numbers and dry weights of the fronds per culture. It was found that in the range of 10 to 100 fronds produced per culture with different nutritional levels, there was a direct relationship with dry weights of the fronds. In N and P nutrition tests 3 or 4 fronds would weigh 1 mg, dry weight, whereas under limiting-Fe conditions about 8 fronds were required to produce 1 mg. The latter effect was very obvious since cultures limited by Fe produced relatively small plants which suggested that limiting-Fe conditions may restrict growth (size of plants) with considerably less influence on multiplication.

If the inoculum for nutrition tests is set at a certain number of fronds per culture, as compared to inoculations with algae based on the number of cells/ml of culture, the volume of the culture will have an influence on the results. In order to demonstrate this factor, nutritional tests were carried out with different N or P concentrations in 25, 50, and 100 ml volumes, using an initial inoculum of 2 fronds per culture. The average numbers of fronds from duplicate cultures are summarized in Table 17.

It can be seen from the data that, with increased volume, more fronds are produced at a constant concentration of nutrient. The effect on nutritional bioassays would be that 0.25 mg N/l could be detected in 50 and 100 ml volumes, but 1 mg N/l would be needed to cause a twofold increase over control (-N) in 25 ml volumes. P concentrations of 0.05, 0.10, and 0.20 mg P/l could be detected in 100, 50, and 25 ml volumes, respectively. It is thus apparent that when using an inoculation of 2 fronds per culture, the volume of the culture must be reported and kept constant for each series of comparative tests.

The previous study indicates how the numbers of fronds produced per culture can be used as an index to the amount of available nutrient present in a culture. Since 3 weeks or more of incubation are required for the maximal growth of cultures, comparative growth tests with different sources of nutrient might not be reliable because of possible conversion of one form of nutrient to another more readily available form over such a long exposure time. Therefore, a series of tests were carried out in which the duckweed was exposed for relatively short periods of time to different sources of nutrients, placed in media lacking the nutrient of interest, and grown on the sorbed nutrient over a 3- or 4-week period. Preliminary tests indicated that comparative tests could be carried out by placing 4 fronds in 50 ml of supplemented Gorham's medium containing either 5 mg N/l, 2.5 mg P/l or 0.025 mg Fe/l, and after different sorption times, transferring 2 fronds each to duplicate 25 ml volumes of Gorham's medium lacking the nutrient of interest. The fronds developed in the media lacking

Table 17. THE EFFECT OF THE VOLUME OF CULTURE MEDIUM ON THE NUMBER OF DUCKWEED (*LEMNA MINOR*) FRONDS PRODUCED WITH DIFFERENT CONCENTRATIONS OF NITROGEN AND PHOSPHORUS
Supplemented Gorham's medium 2 fronds/culture

	Average number fronds/culture after 3 weeks		
Culture medium	25 ml	50 ml	100 ml
Gorham's (-N)	11	11	10
Gorham's (-N) + 0.25 mg NO_3-N/l	12	19	22
Gorham's (-N) + 0.5 mg NO_3-N/l	13	23	36
Gorham's (-N) + 1.0 mg NO_3-N/l	23	40	58
Gorham's (-N) + 2.0 mg NO_3-N/l	30	51	88
Gorham's (-P)	4	3	4
Gorham's (-P) + 0.025 mg PO_4-P/l	5	5	5
Gorham's (-P) + 0.05 mg PO_4-P/l	5	6	9
Gorham's (-P) + 0.10 mg PO_4-P/l	5	17	28
Gorham's (-P) + 0.20 mg PO_4-P/l	22	39	63

the essential nutrient were counted after 3 or 4 weeks of incubation. The results of typical experiments are presented as the average numbers of fronds produced after exposures to different sources of N, P, and Fe, in Tables 18, 19, and 20, respectively.

The data comparing NH_4-N and NO_3-N under these conditions indicate that either source of N was available and required between 6 and 28 hours for maximal sorption to take place. When the different sources of P were compared, ortho- and pyro-P appeared to be sorbed slightly faster than tripoly-P,

Table 18. THE EFFECT OF CONTACT TIME WITH AMMONIUM AND NITRATE NITROGEN ON THE GROWTH OF DUCKWEED (*LEMNA MINOR*)
2 fronds/25 ml Gorham's (-N) medium

Contact time (hours)	Average number of fronds/culture after 4 weeks		
	-N	5 mg NH_4-N/l	5 mg NO_3-N/l
1	5	7	6
2	6	7	7
4	7	7	7
6	8	9	8
28	8	12	11
48	6	14	12

Table 19. THE EFFECT OF CONTACT TIME WITH DIFFERENT SOURCES OF PHOSPHORUS ON THE GROWTH OF DUCKWEED (*LEMNA MINOR*)
2 fronds/25 ml Gorham's (-P) medium

Contact time (hours)	Average number of fronds/culture after 4 weeks			
		2.5 mg P/l		
	-P	ortho	pyro	tripoly
1/2	4	5	4	4
1	3	4	4	3
2	4	5	4	4
4	3	8	6	4
6	4	10	9	6
8	3	12	8	6
24	3	14	26	12
28	4	28	14	17
48	4	17	16	17
52	5	27	27	22

Table 20. EFFECT OF CONTACT TIME WITH DIFFERENT SOURCES OF IRON ON THE GROWTH OF DUCKWEED (*LEMNA MINOR*)

2 fronds/25 ml Gorham's (-N, -EDTA) medium

Contact time (hours)	-Fe	$FeCl_3$ 0.025 mg Fe/l	$FeCl_3$ 0.100 mg Fe/l	1 mg/l citric acid 0.025 mg Fe/l	1 mg/l EDTA 0.025 mg Fe/l
1	3	4	5	5	6
4	5	4	4	4	6
8	4	4	4	6	10
24	4	6	6	4	10
28	5	6	6	3	16
48	6	4	4	7	14
51	4	4	4	5	14
68	6	7	5	5	16

but duckweed could utilize tripoly- as well as ortho- or pyro-P when enough exposure time was allowed. The tests with different sources of Fe indicated that duckweed could only utilize the Fe in the presence of EDTA, with maximal growth occurring after about 1 day exposure. These results illustrate that short-term exposure periods for the comparative sorption of essential nutrients can be used in conjunction with the relatively long incubation time required for maximal growth.

When an essential nutrient is to be tested and there is no problem with potential conversion of the nutrient to another form, simple growth bioassays can be carried out in suitable control cultures and with the source of nutrient. The results of a typical experiment testing the availability of the P of lake muds under aerobic conditions are presented in Table 21 as the average number of fronds developed per triplicate culture. In this type of test, 2 fronds were added to 50 ml of supplemented Gorham's medium which contained either lake mud or different concentrations of P. The depth of the cultures under these conditions was such that the roots of the duckweed were touching the lake mud on the bottom of the flasks.

It is evident that under the conditions of this test, a significant increase in frond numbers occurred with a concentration of 0.1 mg PO_4-P/l in the control cultures, but that as much as 7.5 mg P/l in the form of mud did not support as much

Table 21. AVAILABILITY OF THE PHOSPHORUS OF AEROBIC LAKE MUD FOR THE GROWTH OF DUCKWEED (*LEMNA MINOR*)
2 fronds/50 ml   Gorham's medium   3 weeks incubation

Source of phosphorus	Concentration (mg P/1)	Average number of fronds from triplicate cultures
PO_4-P	0.0	4
PO_4-P	0.025	3
PO_4-P	0.05	7
PO_4-P	0.10	19
PO_4-P	0.25	39
PO_4-P	0.50	45
PO_4-P	1.25	75
Lake Wingra mud	0.15	5
Lake Wingra mud	0.75	4
Lake Wingra mud	3.0	4
Lake Wingra mud	7.5	7
Lake Wingra mud + PO_4-P	0.75 + 0.50	24

growth as 0.1 mg PO_4-P/1. When PO_4-P was added to lake muds containing 0.75 mg P/1, there was a significant growth of the duckweed, but not as much as occurred when 0.5 mg PO_4-P/1 were tested in the absence of the mud. In order to demonstrate that insolubility is not the factor involved in this unavailability of the P of aerobic lake muds, a shark tooth, which had been washed in warm running water for 45 days, was added to a P-free culture, which after 14 days of incubation had produced only 3 fronds. After an additional 12-day incubation there were 22 fronds in this culture. Similar tests for the availability to duckweed of the N or Fe of aerobic lake muds indicated that no available N could be detected in as much as 5 ml of thick mud in a 50 ml culture (Table 22), but aerobic muds were a significant source of Fe for duckweed. The results of these tests were similar to results obtained with a variety of algal species.[1]

Table 22. AVAILABILITY OF THE NITROGEN OF AEROBIC LAKE MUD FOR THE GROWTH OF DUCKWEED (*LEMNA MINOR*)
2 fronds/50 ml Gorham's medium 3 weeks incubation

Source of nitrogen	Concentration (mg N/1)	Average frond number per triplicate culture
NO_3-N	0.0	12
NO_3-N	0.25	17
NO_3-N	0.5	24
NO_3-N	1.0	39
NO_3-N	2.0	56
NO_3-N	5.0	78
Lake Wingra mud	(2 ml)	13
Lake Wingra mud	(5 ml)	12
Lake Wingra mud + NO_3-N	(2 ml) + 2.5	24

The previous tests had indicated that the P of a lake mud sample was not available for the growth of duckweed when the roots of the plant could touch the mud under aerobic conditions. The difference between tests with algae and aquatic weeds, however, lies in the fact that aquatic weeds can be tested under conditions where their roots can penetrate an anaerobic environment while the rest of the plant remains in an aerobic environment, such as occurs in normal growth in a lake.

Consequently, an experiment was carried out comparing the availability of the P in lake muds to duckweed floating so far above the mud surface that its roots could only absorb P which first passed through an aerobic column of medium, with the availability to duckweed whose roots could penetrate the mud and presumably extend into any anaerobic environment developed in the mud. Two fronds were placed in 20 cm culture tubes containing Gorham's (-P) medium. Duplicate cultures contained 50 ml of Gorham's (-P) medium or this medium plus 0.005 mg PO_4-P. To four tubes containing 25 ml of Lake Wingra mud (2.0 mg total P/tube), 30 ml of Gorham's (-P) medium were added so the duckweed floated 8 cm above the surface of the mud. The second set of mud tubes contained only 5 ml of Gorham's (-P) medium so the duckweed floated only about 1 cm from the mud surface. The results of the experiment as the average number of fronds per test condition after 6 weeks incubation are graphically summarized in Figure 12.

Little growth (5 fronds) of the duckweed took place in the P-free Gorham's medium, but 27 fronds developed in the presence of 0.005 mg PO_4-P. It is evident that no available P was released to the overlying medium in the case where the mud containing 2 mg of total P was overlain by 30 ml of Gorham's (-P) medium since the duckweed in these tubes had no more growth than those with only Gorham's (-P) medium. There was considerable growth (27 fronds) of the duckweed whose roots penetrated the mud layers which were presumed to have developed an anaerobic environment during the long incubation period (6 weeks). Observations of these cultures for an additional 8 weeks also indicated that, even with additional incubation, the duckweed in cultures in which the roots could not touch the mud layers did not grow any farther.

These tests thus indicate how tests can be carried out to contrast the availability of P in lake muds to algae or floating aquatic weeds *versus* that to aquatic weeds whose roots can penetrate to anaerobic layers of mud. As one would expect from chemical studies of the release of PO_4-P to the overlying aerobic waters of lakes,[2] there is available P in lake muds which are under anaerobic conditions. One should be aware, however, of the fact that PO_4-P released from anaerobic lake muds into an aerobic environment can be competitively sorbed by muds under the aerobic environment.[1]

References

1. Fitzgerald, G. P. Aerobic Lake Muds for the Removal of Phosphorus from Lake Waters. Limnol. and Oceangr. 15:550-555, 1970.

2. Fitzgerald, G. P. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters, Allen, H. E., and J. R. Kramer (ed.). New York, John Wiley and Sons, 1972. p. 147-170.

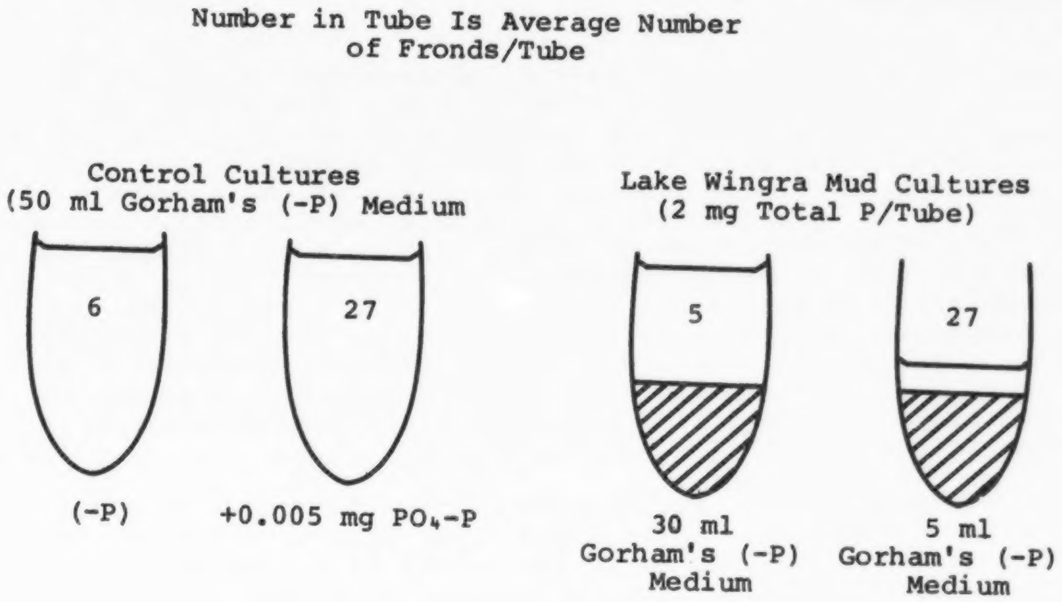
Number in Tube Is Average Number of Fronds/Tube
Control Cultures
(50 ml Gorham's (-P) Medium
6
27
(-P)
+0.005 mg PO₄-P
Lake Wingra Mud Cultures
(2 mg Total P/Tube)
5
27
30 ml Gorham's (-P) Medium
5 ml Gorham's (-P) Medium

Fig. 12. The Availability of Lake Mud Phosphorus for the Growth of Duckweed (*Lemna minor*)
2 Fronds/Tube 6 Weeks Incubation

SECTION VI

CORRELATIONS BETWEEN ALGAL BIOASSAYS AND CHEMICAL ANALYSES TO EVALUATE THE EFFECTS OF WASTEWATER PHOSPHORUS ON RECEIVING WATERS

Introduction

The use of algal growth tests to measure the concentration of a limiting nutrient in a water sample is based on the assumption that, under the conditions of the test, the growth attained by the algae will be directly dependent upon the concentration of the limiting nutrient. Of the nutrients required for plant growth in an aquatic environment, the consensus of ecology-oriented scientists[1,2] is that phosphorus is most able to be controlled to a degree which will allow regulation of its fertilizing effect. In order to get a perspective of the relative amounts of phosphorus available for algal growth contributed by the Madison, Wisconsin area lakes and cities to the Yahara and Rock Rivers, a series of bioassays and chemical analyses of river samples has been carried out.

It was assumed that during late summer the growth of algae in the Madison area lakes would cause them to act as sinks for available phosphorus in the Yahara River system. If the rivers below the lake outlets had an excess of phosphorus during this period, it would indicate that the amount of phosphorus in the system is great enough to provide more phosphorus than the algae in the lakes can utilize. Such a situation existed in Lakes Waubesa and Kegonsa during the early 1940's.[3] While Lake Kegonsa supported about 135 metric tons (dry weight) of algae, its outlet had concentrations of more than 0.4 mg soluble PO_4-P/1.

The main source of nitrogen and phosphorus to these lakes was the secondary effluent of the Madison Sewage Treatment Plant, which entered the Yahara River just above Lake Waubesa. In 1958, however, the effluent from the treatment plant was diverted around Lakes Waubesa and Kegonsa and piped to the

Badfish Creek, which enters the Yahara River system below the City of Stoughton. It is, therefore, possible to test for differences in the available phosphorus concentrations below each lake and the City of Stoughton, evaluate the relative contribution of these sources, and determine whether the effluent from the Madison Sewage Treatment Plant can have any influence on the phosphorus economy (phosphorus available *versus* phosphorus needed for algal growth) of the Yahara River system. In turn, the potential effect of the available phosphorus contributed to the Rock River by the Yahara River can be evaluated. (A general diagram of the Madison lakes, Yahara River-Rock River system is presented in Figure 13.)

Materials and Methods

In order to determine whether the ortho or the total phosphorus of the Yahara and Rock Rivers is available for the test algae, and whether the system contains surplus available phosphorus upstream from the sewage effluents of the surrounding cities, the present study has utilized two types of algal bioassays and chemical analyses: short-[4] and long-term[5] bioassays to measure the available phosphorus in water samples, *in situ* bioassays to determine limiting or surplus phosphorus conditions,[6] and chemical analyses of soluble ortho (stannous chloride method)[7] and total phosphorus.[8] The short-term bioassay requires incubation for 1 day, in samples to be tested, of small quantitites of P-limited *Cladophora* sp or *Rhizoclonium* sp collected from Madison area lakes. The amount of extractable PO_4-P from algae after incubation in known concentrations of PO_4-P is compared with the amount obtained from algae after incubation in the different water samples, and is used to calculate the concentrations of available phosphorus in the water samples. The results of a typical test in which *Cladophora* sp from the 3-meter depth of Lake Mendota was incubated in different concentrations of ortho PO_4-P in Gorham's medium[9] are presented in Table 23 as the concentrations of extractable (one hour, boiling water bath) PO_4-P from the algae. Similar direct relationships between concentrations of available phosphorus and the concentrations of phosphorus in algae can be obtained with total phosphorus analyses of the algae. Since such tests merely measure the portion

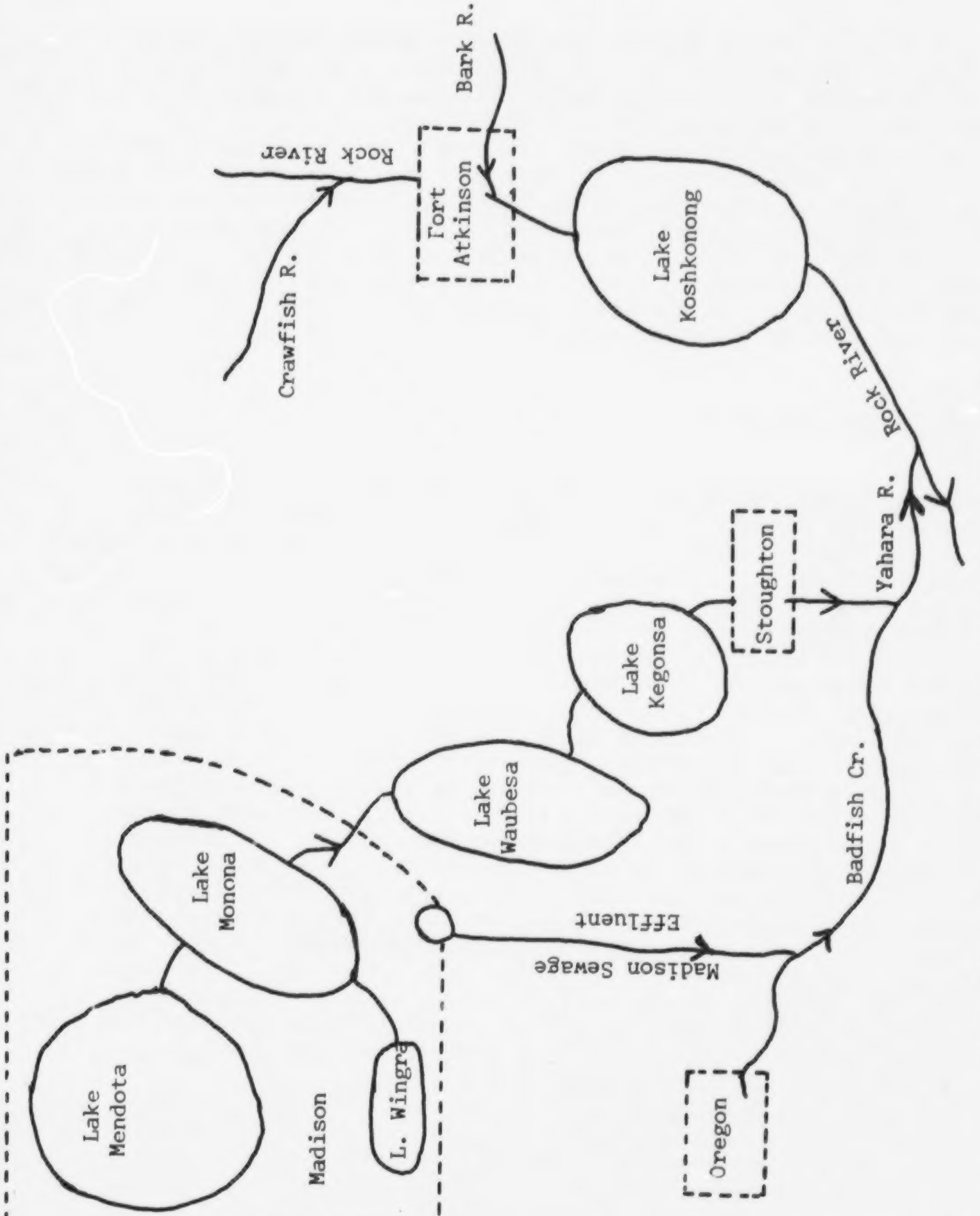
Lake Mendota
Madison
L. Wingra
Lake Monona
Madison Sewage Effluent
Oregon
Lake Waubesa
Badfish Cr.
Lake Kegonsa
Stoughton
Yahara R.
Rock River
Lake Koshkonong
Fort Atkinson
Bark R.
Rock River
Crawfish R.

Fig. 13. General Diagram of the Madison Lakes, Yahara River-Rock River System

Table 23. THE EXTRACTABLE PO_4-P
OF *CLADOPHORA* SP INCUBATED ONE DAY
IN DIFFERENT CONCENTRATIONS OF PHOSPHORUS
10 mg samples of *Cladophora* from Lake Mendota
per 500 ml Gorham's (-P) medium
One hour boiling water extraction

Phosphorus concentration in medium (mg P/l)	Extractable PO_4-P (mg P/100 mg algae)
none	0.025
0.04	0.076
0.08	0.116
0.12	0.162

of phosphorus that the algae sorb from the test waters over a 1-day period, a test to determine the amount of growth sustained by the phosphorus in the samples has been used. By adding *Selenastrum capricornutum* (AAP)[5] at a final concentration of 1,000 cells/ml to control cultures of AAP medium containing known levels of PO_4-P and to the test waters, a comparison of the growth attained in the respective cultures can be made, and the concentrations of available phosphorus in the test water can be calculated. Because of the fact that in some lake and river samples phosphorus is not the limiting nutrient, in order to allow the algae to utilize all of the phosphorus available for growth, the sample frequently needs to be supplemented with nitrogen and iron. Therefore, some samples have 2 mg NO_3-N/l, 0.03 mg Fe plus 0.3 mg EDTA/l, or both, added.

The sensitivity of the method used to measure the growth attained will determine the length of incubation required and the range of concentrations of the nutrient over which the measurable growth of the algae will respond in a straight line relationship. Cell counts with an electronic particle counter or chlorophyll *a* measurements with a fluorometer are relatively sensitive in comparison to cell counts with a haemocytometer slide and microscope, absorbance, or dry weight measurements. Examples of typical data are presented in Table 24 as the growth of *Selenastrum capricornutum* (AAP) in different concentrations of ortho PO_4-P in AAP medium[5] as measured by the *in vivo* fluorescence of chlorophyll *a* after 5 days and cell counts after 12 days. Either method gives a direct relationship between growth attained and the concentration of phosphorus in the culture medium. The results of the two types of bioassays when compared to chemical analyses will indicate whether the ortho or total phosphorus of the sample water is available for the algae tested. The phosphorus nutritional status of *in situ* algae can be determined by sample analyses similar to those used in the short-term bioassay. Collections of algae are taken from the test lake

Table 24. THE GROWTH OF *SELENASTRUM CAPRICORNUTUM* (AAP) IN DIFFERENT CONCENTRATIONS OF PHOSPHORUS IN AAP MEDIUM

Phosphorus concentration (mg P/1)	Average growth attained: After 5 days *in vivo* chlorophyll *a* (fluorescence units)	Average growth attained: After 12 days cell counts (cells/ml x 10^{-3})
none	9	7
0.025	223	180
0.05	630	360
0.075	880	480
0.10	1,260	600

or river and extracted for PO_4-P to determine if they had limiting or surplus phosphorus at the time of sampling. It has been shown that algae that are phosphorus-limited will contain low concentrations of extractable PO_4-P (less than 0.08 mg P/100 mg algae)[5] or total phosphorus.[10] Therefore, these relatively simple analyses can be used to compare the levels of phosphorus available to existing algal populations in different environments or after different environmental changes.

Results

Sources of phosphorus in the Yahara River -

Surveys of the phosphorus content of the Yahara River have been carried out by comparing the results of bioassays for available phosphorus (using both the one-day sorption-extraction test and the long-term growth test) with chemical analyses of soluble PO_4-P and total phosphorus.

Water samples were collected at two different times in September of 1971 from the Yahara River below Lake Waubesa, Lake Kegonsa, and the City of Stoughton and from the Badfish Creek below the City of Oregon (containing combined effluents from the sewage treatment plants of Madison and Oregon). *Rhizoclonium* sp from the 3-meter depth of Lake Mendota was used in a 22-hour sorption-extraction bioassay for available phosphorus, and *Selenastrum capricornutum* (AAP) was used in a long-term (9-day) growth test. The extractable PO_4-P from samples of *Rhizoclonium* (16 mg samples/500 ml) incubated over night in controls with known concentrations of phosphorus were used to calculate the concentrations of available phosphorus in the test waters. The results of these calculations are presented in the first column of Table 25.

The amount of growth supported by the phosphorus of the three Yahara River samples was also tested by adding *Selenastrum* at a final concentration of 1,000 cells/ml to control cultures of AAP medium containing different levels of PO_4-P and to the river waters. Since preliminary tests had indicated that the samples taken from the stream below Lake Kegonsa and the City of Stoughton contained relatively high phosphorus concentrations, these samples were diluted to 50% and 20%, respectively, (double-distilled water with 15 mg $NaHCO_3$/l). Some samples were supplemented with 2 mg NO_3-N/l, 0.03 mg Fe plus 0.3 mg EDTA/l, or both. Growth of *Selenastrum* in the cultures was followed, and the maximum growth was shown to have been reached after 9 days incubation.

Table 25. COMPARISONS OF PHOSPHORUS ANALYSES OF YAHARA RIVER AND BADFISH CREEK: BIOASSAYS *VERSUS* CHEMICAL ANALYSES

	Phosphorus concentration (mg P/l)			
	Bioassays		Chemical analyses	
Sample	1-day sorption and extraction	9 days growth	Soluble PO_4-P	Total P
Yahara River below Lake Waubesa	0.07	0.10	0.054	0.14
Yahara River below Lake Kegonsa	0.12	0.13	0.11	0.23
Yahara River below City of Stoughton	0.27	0.28	0.28	0.40
Badfish Creek below City of Oregon	8.0	-	7.7	8.0

The growth of *Selenastrum* increased with increases in PO_4-P in the control cultures, indicating that the test alga was able to respond to different levels of phosphorus. The results of the tests in the river waters showed that growth was increased by the addition of nitrogen or nitrogen plus iron. The maximum level of growth in the supplemented river samples was used to calculate the amount of available phosphorus in the samples, by reference to the growth attained with known concentrations of phosphorus (second column, Table 25).

The calculated concentrations of available phosphorus from the *Selenastrum* growth test are compared to results from the one-day bioassay and chemical analyses in Table 25. These data indicate that the two bioassays for available phosphorus agree very well with each other and with the analyses for soluble PO_4-P. The total phosphorus analyses of these samples were not sufficiently different from the soluble PO_4-P analyses to contrast with the bioassays.

In addition to water from the different river stations sampled for bioassays and chemical analyses, collections of *in situ* algae were made from the Yahara River below Lake Waubesa and Lake Kegonsa. These algae were extracted for PO_4-P to determine if they had limiting or surplus phosphorus at the time of sampling. *Cladophora* sp collected below Lake Waubesa had 0.12 mg extractable PO_4-P/100 mg algae, while the water sample contained 0.05 mg PO_4-P/l. *Spirogyra* sp from below Lake Kegonsa had 0.49 mg PO_4-P/100 mg, and the water sample contained 0.1 mg PO_4-P/l. In contrast, *Rhizoclonium* sp collected the same day from the 3-meter depth of Lake Mendota had 0.075 mg extractable PO_4-P/100 mg algae, correlating with less than 0.02 mg PO_4-P/l in the surface waters of that lake.

The data from bioassays for *in situ* extractable PO_4-P, growth experiments, and chemical analyses indicate that the Yahara River below Lake Kegonsa contained available P in excess of that required by the lake algae during the late summer of 1971. Since there was considerably more P leaving Lake Kegonsa than Lake Waubesa, it appears that Lake Kegonsa and its drainage area increase the phosphorus content of the Yahara River to such an extent that phosphorus is present in the lake in excess of that which can be utilized by the dense growths of algae or aquatic weeds present, and this situation has not changed since 1943 despite the diversion around Lakes Waubesa and Kegonsa of the secondary effluent of the Madison Sewage Treatment Plant in 1958.

Comparison of the phosphorus contents of the outlets of the Madison lakes -

Inasmuch as the tests in the Yahara River had indicated that surplus phosphorus (0.12 mg PO_4-P/l) was leaving Lake Kegonsa during a period of the year when it would be expected that algae would be reducing the phosphorus content of lake waters to minimal concentrations, a comparison was made by bioassay and chemical analyses of the phosphorus leaving all five of the Madison area lakes during the late summer of 1971.

Two tests were carried out for the available phosphorus concentration in the lake waters by the sorption-extraction bioassay. In one test *Cladophora* sp from Lake Mendota was used, and in the other *Cladophora* sp from Lake Wingra was used. Duplicate 5-10 mg samples were incubated 20 hours in 500 ml of the control and test waters. The algae were then rinsed and extracted for PO_4-P. The results of the test with Lake Mendota *Cladophora* are summarized in the first column of Table 26 as the calculated concentrations of available phosphorus obtained from comparisons of extracts of algae from control and test waters.

Table 26. COMPARISON OF THE PHOSPHORUS CONTENTS OF THE OUTLETS OF MADISON AREA LAKES: BIOASSAY *VERSUS* CHEMICAL

Lake outlet	Bioassay		Chemical	
	1-day sorption and extraction	10 days growth (AAP)	Soluble ortho PO_4	Total
Mendota	0.011	0.014	0.01	0.05
Wingra	0.010	0.030	0.02	0.05
Monona	0.005	0.024	0.02	0.05
Waubesa	0.030	0.041	0.050	0.090
Kegonsa	0.10	0.11	0.12	0.15

It is evident that the samples from the outlets of Lakes Mendota, Wingra, and Monona contained barely detectable available phosphorus. There was considerably more available phosphorus in the sample from Lake Kegonsa than from Lake Waubesa (0.10 mg *versus* 0.03 mg P/l). Similar results were obtained when *Cladophora* from Lake Wingra was used.

The growth of *Selenastrum* after 10 days incubation in the lake outlet samples supplemented with nitrogen and iron was also used to calculate the concentrations of available phosphorus. These results are summarized in the second column of Table 26. The value reported for the Lake Wingra sample (0.03 mg P/l) calculated from cell count data is significantly higher than that calculated from fluorometric measurements of chlorophyll *a* (0.018 mg P/l). The latter value is more in line with the relatively low concentration of soluble PO_4-P found by chemical analysis (Table 26). In general, the concentrations of available phosphorus correlate well with the concentrations of soluble PO_4-P of these samples. Since these samples contain little phosphorus that is not soluble PO_4-P, no further correlations were made.

A comparison of the extactable PO_4-P and total phosphorus of algae collected from the outlets of the different lakes has also been made as a measure of the phosphorus available to *in situ* algae. *Cladophora* sp were collected from all environments, and *Spirogyra* sp from two areas. The results of extractions of PO_4-P or of the total phosphorus of the *in situ* algae are presented in Table 27.

Table 27. PHOSPHORUS CONTENT OF *IN SITU* ALGAE COLLECTED FROM THE OUTLETS OF THE MADISON LAKES

	Averages of 2 to 6 samples (mg P/100 mg algae)		
	Extractable PO -P		Total P
Source	*Cladophora* sp	*Spirogyra* sp	*Cladophora* sp
L. Mendota	0.065	-	0.16
L. Monona	0.066	-	0.16
L. Wingra	0.038	-	-
L. Waubesa	0.084	0.14	0.21
L. Kegonsa	0.14	0.40	0.28

The data on the amounts of extractable PO_4-P of the *Cladophora* from the lake outlets indicate that algae from Lake Kegonsa had relatively high values compared to the algae from the other lakes. The extractable PO_4-P of the *Spirogyra* samples from the outlet of Lake Kegonsa also indicated that algae from this environment had considerably more phosphorus available to them than to the *Spirogyra* from the outlet of Lake Waubesa. Analyses of the total phosphorus of the *Cladophora* indicate that algae from Lakes Mendota and Monona had similar phosphorus contents, but *Cladophora* from Lakes Waubesa and

Kegonsa had increased phosphorus contents, the algal phosphorus concentration found at the outlet of Lake Kegonsa being nearly double that at Lakes Mendota and Monona.

It is evident that, in the late summer, the phosphorus concentration and eutrophication level of water leaving the three lakes surrounded by the City of Madison is considerably less than that of Lake Kegonsa, with Lake Waubesa having an intermediate level.

The phosphorus content of the Rock and Yahara Rivers -

There has been some conjecture as to the effect of the phosphorus from the Madison Sewage Treatment Plant on the phosphorus economy of the Rock River. In order to establish an effect, it would be necessary to know if any available phosphorus was present in the Rock River above the junction with the Yahara River which brought the Madison Sewage Treatment Plant effluent from the Badfish Creek. Samples for analyses were taken at two stations on the Rock River above the junction with the Yahara, two stations on the Yahara River after it received the waters of the Badfish Creek, and one station on the Rock River below the junction with the Yahara River. Two 24-hour bioassays were carried out using *Cladophora* sp from Lake Mendota to sorb the available phosphorus from the test waters. One test measured the phosphorus sorbed by the extraction of PO_4-P and the other test measured the total phosphorus of algae after incubation. In addition, growth tests with *Selenastrum* were carried out. Thus, the results of 3 types of bioassays could be correlated with chemical analyses for soluble PO_4-P and total phosphorus of the test waters. The results of these tests are summarized in Table 28.

The analyses for soluble PO_4-P of the samples used in the growth experiment are reported separately since there was a general increase after the samples were processed for this test. The growth test samples were autoclaved, gassed with CO_2 to dissolve any precipitate formed, aerated to remove excess CO_2 and raise the pH back to normal, and then filtered (Whatman #2) to remove any precipitates that would interfere with the electronic particle counting instrument used in measuring the growth of the *Selenastrum*.

The analyses of samples of Rock River water upstream from the junction with the Yahara River show that this river contains

Table 28. THE PHOSPHORUS OF THE ROCK AND YAHARA RIVERS: BIOASSAYS *VERSUS* CHEMICAL ANALYSES

	Phosphorus concentration (mg P/l)				
	Bioassays-available phosphorus			Chemical analyses	
	1-day sorption		10 days growth (AAP)		
Samples	Extractable PO_4-P	Total P		Soluble PO_4-P	Total P
Rock River Newville-above Yahara River	0.11	0.090	0.25	0.14 (0.28)[a]	0.50
Rock River Indianford-above Yahara River	0.10	0.060	0.30	0.17 (0.23)	0.41
Rock River U.S. 14-below Yahara River	0.56	0.65	0.86	0.77 (0.96)	1.2
Yahara River Wis 59-below Badfish Creek	0.51	0.60	1.2	0.62 (1.0)	1.2
Yahara River Fulton-below Badfish Creek	1.4	0.95	1.5	1.9 (1.9)	2.6

[a] Analyses in parentheses are of soluble PO_4-P of samples for AAP growth tests

about 0.1 mg available P/l even before the junction with the Yahara River. The effect of the Yahara River on the phosphorus content of the Rock River is shown by the increase in available phosphorus to more than 0.5 mg/l. The differences in the samples collected from the Yahara River probably reflect the fact that the sample collected at Highway 59 was close to the junction with the Badfish Creek and may not have been as well mixed with the Badfish Creek water as the sample collected farther downstream (Town of Fulton).

The phosphorus content of the Rock River and two of its tributaries -

In order to determine the source of the phosphorus found in the Rock River above the junction with the Yahara River, upstream samples of the Rock River and two of its tributaries were tested with bioassays and chemical analyses. The Rock River was sampled at County Highway B above the City of Jefferson and at Wisconsin Highway 59 below Lake Koshkonong. The Crawfish and Bark Rivers were sampled near their junctions with the Rock River. Therefore, the amount of phosphorus in the Rock River above the Cities of Jefferson and Fort Atkinson could be compared with the phosphorus in the river below these stations. It was also possible to test if the plankton and aquatic weeds of Lake Koshkonong caused the lake to act as a sink for the phosphorus of the Rock River.

The phosphorus sorbed by P-limited *Cladophora* sp from Lake Wingra was measured as extractable PO_4-P after 22 hours incubation in control samples with known concentrations of phosphorus and in dilutions of the field samples. The results of the tests were used to calculate the available phosphorus concentrations in the river water samples (2nd column, Table 29).

Table 29. THE PHOSPHORUS OF THE ROCK RIVER AND SOME TRIBUTARIES: BIOASSAYS *VERSUS* CHEMICAL ANALYSES

	Phosphorus concentration (mg P/l)			
	Bioassays		Chemical analyses	
Sample	1-day sorption	5 days growth	Soluble PO_4-P	Total P
Rock River at Co B	0.28	0.18	0.23	0.37
Crawfish River	0.22	0.28	0.21	0.44
Bark River	0.65	0.57	0.55	0.92
Rock River at Wis 106	0.50	0.57	0.51	0.68
Rock River at Wis 59	0.10	0.19	0.14	0.30

The growth of *Selenastrum* in known concentrations of phosphorus and in dilutions of the river water samples was measured after 5 and 12 days by cell counts and chlorophyll *a* measurements (fluorometry). From the results of the cell count data after 5 days growth, calculations were made of the available phosphorus in the original river water samples and are presented in the third column of Table 29.

Comparisons of the data from the two bioassays and chemical analyses of soluble PO_4-P indicate that all three analyses agree quite well. The concentrations of phosphorus present in the different samples indicate that there is available phosphorus in the Rock River and its two tributaries, the Crawfish and Bark Rivers, even before the sewage effluents of the Cities of Jefferson and Fort Atkinson are added to the Rock River. The 0.5 mg PO_4-P/l in the Rock River below Fort Atkinson and above Lake Koshkonong can be contrasted with the 0.1 mg PO_4-P/l in the Rock River below Lake Koshkonong. Although the lake apparently acts as a sink for the phosphorus of the Rock River at this time of year (September, 1971), the river below the lake still contains phosphorus in excess of that required for growth of the algae and aquatic weeds in the lake, in contrast to the situation in the case of the three lakes in the Madison area, Lakes Mendota, Monona, and Wingra.

Conclusions

Although only a limited number of field samples contained total phosphorus that did not analyze as soluble ortho PO_4-P, it is evident that soluble ortho PO_4-P analyses correlate with bioassays for available phosphorus and total phosphorus analyses do not, indicating that either method (bioassay or chemical ortho PO_4-P analysis) could be used to measure the phosphorus available for the growth of algae.

There was close agreement of results between the two types of bioassay: the one-day sorption-extraction test *versus* the long-term growth assay, even though the two methods differ greatly. While the one-day sorption-extraction technique relies on mere sorption, by a relatively large amount of algae, of the phosphorus from the medium, the growth test requires that nitrogen or iron-EDTA, or both, be added to some test waters for the minute inoculum (1,000 cells/ml) of *Selenastrum* to grow and utilize all of the available phosphorus. Thus, the longer incubation times and the need to supplement certain samples with additional nitrogen or nitrogen and iron in order for the algae to utilize all of the available phosphorus for growth can be eliminated in most surveys for sources of available phosphorus in natural waters. However, since the growth of algae on the phosphorus in water samples is the ultimate proof of the availability of phosphorus, the longer growth tests must still be used occasionally to check on the results obtained with the short-term bioassays or chemical analyses of soluble ortho PO_4-P.

The tests on the Yahara River have shown that the water leaving the lakes in the City of Madison, Lakes Mendota, Monona, and Wingra, is relatively free of available phosphorus during the late summer period when the algae and aquatic weeds would be expected to have used what phosphorus was available. Therefore, despite the large urban influence on Lakes Mendota, Monona and Wingra, the phosphorus supplied to these lakes apparently is held within these lakes and does not appear as surplus phosphorus in the lake outlets during the peak periods of aquatic plant production. In contrast, Lake Waubesa and especially Lake Kegonsa have such large sources of phosphorus that measurable quantities escape from the lakes as surplus phosphorus in the lake outlets. Therefore, the fact is that the Yahara River contains surplus phosphorus before the influence of the sewage effluents of the Cities of Stoughton, Oregon, and Madison can become apparent. Thus, there are significant sources of available phosphorus in Lakes Waubesa and Kegonsa other than the Yahara River. In order to reduce the available phosphorus concentration in the Yahara River it would be as important to control these sources of available phosphorus as the phosphorus from the sewage effluents of the Cities of Stoughton, Oregon, and Madison.

Speculations on the sources of phosphorus to Lakes Waubesa and Kegonsa frequently mention the fact that for many years these lakes were fertilized by the sewage effluent from the City of Madison. A portion of this phosphorus was deposited in the bottom of the lakes and could be a potential source of phosphorus to the lake waters even after the sewage effluent was diverted around these lakes in 1959. It would be expected that the effect of phosphorus from this source would be most pronounced in Lake Waubesa because this lake received the sewage effluent directly from the treatment plant. However, the fact that more phosphorus is found in the outlet of Kegonsa than of Lake Waubesa indicates that the phosphorus deposited in the lake muds during the years of heavy fertilization by the Madison sewage effluent must not be as important a source of phosphorus as others that continue to add phosphorus at the present time. Swingle *et al*[11] have presented good evidence that phosphorus deposited in muds of shallow lakes is not an available source of fertilizer. Fish ponds that had been heavily fertilized for 15 years with N-P-K fertilizers to maintain productivity of crops of fish were tested to determine which fertilizers could be regenerated from the very fertile bottom muds. It was found that no significant decrease in production of fish resulted from omitting both nitrogen and potassium from the fertilizer mixture during a four-year experimental period. Omission of phosphorus, however, caused a decrease in production. Thus, except for phosphorus, adequate nutrients were available from these fertile pond bottoms. Therefore, the important sources of phosphorus that are supplying surplus quantities of phosphorus to Lakes Waubesa and Kegonsa are probably not related to past fertilization of these lakes but are the result of present sources of phosphorus. The effect of interceptor sewers (under construction) partially around Lake Waubesa may indicate the contribution of phosphorus from the many homes along the lakeshore. Further study of the Lake Kegonsa area may indicate the relative importance of developing a sewer system *versus* directing around the lake a creek that is known to carry a relatively high load of nutrients, as proposed in the 1944 study of Sawyer, Lackey and Lenz.[3]

The Rock River and two of its tributaries, the Crawfish and Bark Rivers, contain available phosphorus above Lake Koshkonong (more than 0.2 mg P/l). In addition, the Rock River contains more than 0.1 mg available P/l below the lake and above its junction with the Yahara River, which indicates that the Rock River already contains phosphorus in excess of that which could be used by the algae and aquatic weeds of the river or Lake Koshkonong, even before the introduction of the effluents from Stoughton, Oregon, and Madison. Therefore, although the Yahara River adds available phosphorus to the Rock River, the significance of this phosphorus is questionable since the river already contains more phosphorus than can be used by the plants present during this time of the year (September, 1971).

The data from bioassays and chemical analyses presented indicate how nutrient surveys can be used to determine whether lakes contain available phosphorus in excess of that which could be used by the algae or aquatic weeds present. If a lake is shown not to have surplus available phosphorus during the summer period, as in the case of the lakes in the City of Madison, Lakes Mendota, Monona, and Wingra, it would be expected that further reductions in the sources of phosphorus to these waters might reduce the biomass of algae present. When a lake contains surplus phosphorus for the amount of algae and aquatic weeds present, it is important to determine the major sources of phosphorus in order to distinguish between those that can be economically reduced from the sources that are unmanageable. Only with such information can a realistic evaluation of a situation be made before costly phosphorus removal programs are undertaken.

References

1. Nutrients and Eutrophication: The Limiting Nutrient Controversy. Symposium, American Society of Limnology and Oceanography, Hickory Corners, Michigan, 1971. W. K. Kellogg Biological Station, Michigan State Univ.

2. Massey, A., and J. Robinson. A Review of the Factors Limiting the Growth of Nuisance Algae. Water and Sewage Works 118:352-355, 1971.

3. Sawyer, C. N., J. B. Lackey, and A. T. Lenz. Investigation of the Odor Nuisance Occurring in the Madison Lakes. Madison, Wis. Governor's Committee Report. 1944. 137 p.

4. Fitzgerald, G. P. Aerobic Lake Muds for the Removal of Phosphorus from Lake Muds. Limnol. and Oceanogr. 15:550-555. 1970.

5. U.S. Environmental Protection Agency. Algal Assay Procedure: Bottle Test. Corvallis, Oregon. Environmental Protection Agency. 1971.

6. Fitzgerald, G. P., and T. C. Nelson. Extractive and Enzymatic Analyses for Limiting or Surplus Phosphorus in Algae. J. Phycol. 2:32. 1966.

7. American Public Health Association. Standard Methods for Water and Wastewater. 12th edition. New York. Amer. Public Health Assoc. 1965.

8. Gales, M. E., Jr., E. C. Julian, and R. C. Kroner. Method for Quantitative Determination of Total Phosphorus in Water. J. Amer. Water Works Assoc. 58:1363. 1966.

9. Hughes, E. P., P. R. Gorham, and A. Zehnder. Toxicity of a Unialgal Culture of *Microcystis aeruginosa*. Can. J. Microbiol. 4:225. 1958.

10. Gerloff, G. C. Evaluating Nutrient Suppleis for the Growth of Aquatic Plants in Natural Waters. In: Eutrophication: Causes, Consequences, Correctives. Washington, D.C., Nat. Acad. Sci., 1969. p. 537-555.

11. Swingle, H. S., B. C. Gooch, and H. R. Rabanal. Phosphate Fertilization of Ponds. In: Proc. 7th Annual Conference, S.E Assoc. Game and Fish Comm., Hot Springs, Oct. 1963. p. 213-218.

SECTION VII

COMPARATIVE CHEMICAL AND BIOASSAY ANALYSES OF THE NUTRIENTS IN THE MADISON AREA LAKES

Introduction

The Madison area lakes, Lakes Mendota, Monona, Wingra, Waubesa, and Kegonsa, have had a well documented history of being troubled by algal and weed problems during the warm seasons when the lakes also have the most appeal as recreational sites for the general public. The displeasure of the public with the conditions of the lakes has resulted in a series of projects for the reversal of the eutrophication of the lakes. These have included the diversion of treated sewages from Lake Monona and the diversion of metropolitan Madison's treated sewage from Lakes Waubesa and Kegonsa. Thus, there has been a gradual reduction of man's influence on these lakes. At the present time, there is a unique set of circumstances taking place which allow us to evaluate the effect of certain of man's influences on lakes with past problem-clouded histories.

The Madison area lakes have a drainage area of 500 to 550 square miles of fertile farm lands, wooded slopes, and urban areas. The various sewage diversion projects evaluated in earlier reports[1] have lessened the influence of man to the extent that the sewage of a population of more than 200,000 people in the watershed of Lakes Mendota, Monona, and Wingra has been diverted around all of the area lakes. The lakes continue to have both algal and weed problems, so further diversion projects have been initiated by local populations. We now have an opportunity to study and record the effect of these new projects so that the end result will be the relative effect of 500 to 550 square miles of fertile farm land drainage *versus* the sewages of various local populations, some areas being sewered and some areas using septic tank disposal. This unique situation will take place in a series of steps that will allow for the collection of present-condition data before each change takes place.

The nutrient control projects in the Madison area are: 1) diversion of the sewage effluents of the Villages of Waunakee and DeForest (accounting for 10% of the inorganic nitrogen and 36% of the phosphorus entering Lake Mendota) from the Lake Mendota watershed to a metropolitan sewer system by-passing the Madison area lakes (1971); 2) collection of sewages from cottages and homes (presently using septic tanks) surrounding Lake Waubesa and disposal to the by-pass sewer system: construction started in 1972 and will be conducted in two stages, each affecting different populations; and 3) collection, treatment, and by-pass disposal of sewages of the cottages and homes (septic tank disposal) of the Lake Kegonsa area: a sanitary district has been created, but construction has not been started.

Studies have been made by the principal investigator and others associated with the Water Chemistry Program of the University of Wisconsin, using chemical and bioassay techniques, to follow the changes in the nitrogen and phosphorus nutritional status of algae in the surface waters of Lakes Mendota and Monona throughout the algal-growing seasons of 1968 to the present.[2,3,4] These data can be used to compare with results in 1973 and later, to determine if any changes can be correlated with the diversion around the Madison lakes of the sewage effluents of the Villages of Waunakee and DeForest in contrast to the continuing effects on this deep, stratified lake caused by drainage from 250 square miles of fertile farm area.

Data collected in September, 1971[4] indicated that, before the sewers were built around Lake Waubesa, the concentration of soluble PO_4-P and available P was 0.05-0.07 mg P/1 at the outlet of Lake Waubesa. However, after the sewer construction of 1972, the levels of soluble PO_4-P and available P in Lake Waubesa did not exceed 0.03 mg P/1 throughout August and September of 1972. Thus, there is an indication that the conversion of septic tanks to sewerage and diversion had a significant effect on the level of P available in Lake Waubesa despite normal runoffs from the fertile farm lands of the area. Further data from this area as the sewerage program continues should substantiate the results obtained in 1972.

The results of analyses of Lake Kegonsa and its algae provide spectacular data, since it is the only lake of the five-lake chain which had increasing levels of soluble PO_4-P and available P during July, August, and September (to more than 0.1 mg P/1) when the other lakes were practically devoid of soluble PO_4-P. Thus, urban and farm land surface runoffs were not providing surplus P to the other lakes at a time Lake Kegonsa was obtaining P above the concentration its aquatic plants could utilize. This increase in P in Lake Kegonsa is therefore believed to be due to septic tank drainage. Confirmation of these data during 1973 will provide a firm basis for evaluating the proposed sewerage of the Lake Kegonsa area population and a separation of the effects of farm runoff in this very fertile area *versus* the effect of septic tank drainage. Since the levels of P in this lake are so spectacular before the sewerage system (0.1 mg P/1), it should provide a very visible test of the effect of cottage population sewerage programs on the level of eutrophication of a lake.

Application of Algal Assay Procedures

Comparative AAP tests using *Selenastrum*, *Microcystis*, and *Anabaena* have been carried out in water samples from the outlets of the five Madison area lakes, Mendota, Monona, Wingra, Waubesa, and Kegonsa. All the lakes were covered with ice at the sampling times. Two samples were tested in January, 1973 when there were high flows due to rain and snow melts, and two samples were tested in February, 1973 during a period of subfreezing weather when there were no surface runoffs. The maximum growth attained by the algae added to the lake waters was measured as *in vivo* chlorophyll *a* using a fluorometer. The instrument used was most sensitive to the chlorophyll of *Selenastrum* so, at about equal dry weight concentrations, the relative amounts of chlorophyll measured were 10, 1, and 1.5 for *Selenastrum*, *Microcystis*, and *Anabaena*, respectively. The growth of the algae in all lake waters which were spiked with a combination of N, P, and Fe (NAAM level) was relatively high so algal growth attained was dependent upon: the lake tested, Mendota usually supporting maximal growth and Wingra supporting the least growth; whether the samples were collected during high or low flows; and what spikes of N, P, Fe, or combinations were added. The relative growths of the algae are presented in Table 30 as maximal fluorometry units attained in the autoclaved water samples and in the presence of added N and Fe.

The data show that the growth of *Selenastrum* was most consistent in similar samples and is the alga of choice for most growth tests using these three algae because, even with inocula of 1,000 cells per ml compared to 50,000 cells per ml for the other algae, the maximal growth was attained in 7 to 9 days whereas the other algae usually required 2 weeks or more. The results of the tests with no added spikes of

Table 30. THE ALGAL GROWTH ATTAINED, AS FLUOROMETRY UNITS, IN AUTOCLAVED SAMPLES OF MADISON AREA LAKE WATERS
Averages of triplicate cultures

	No spikes				NAAM level of N and Fe added			
	High flows		Low flows		High flows		Low flows	
	1/18/73	1/30/73	2/12/73	2/22/73	1/18/73	1/30/73	2/12/73	2/22/73
Selenastrum (1,000 cells/ml)								
Mendota	1.5	1.6	0.1	0.2	7.8	8.7	4.1	4.7
Monona	0.8	0.9	0.4	0.3	5.4	5.5	3.8	5.4
Wingra	0.04	0.02	0.02	0.05	0.06	0.03	0.09	0.05
Waubesa	1.1	1.2	1.3	1.2	3.0	4.0	3.6	3.5
Kegonsa	0.7	0.4	0.3	0.9	0.8	0.6	1.3	1.3
Microcystis (50,000 cells/ml)								
Mendota	0.05	0.03	0.02	0.1	1.6	1.8	0.7	0.7
Monona	0.03	0.03	0.03	0.06	0.9	1.0	0.7	1.0
Wingra	0.04	0.1	0.03	0.08	0.05	0.2	0.09	0.2
Waubesa	0.04	0.04	0.04	0.03	0.5	0.6	0.5	0.7
Kegonsa	0.04	0.05	0.05	0.1	0.1	0.4	0.2	0.4
Anabaena (50,000 cells/ml)								
Mendota	0.7	1.0	0.03	0.6	1.9	1.5	0.08	1.1
Monona	0.7	0.4	0.07	0.5	1.3	1.5	0.06	1.0
Wingra	0.06	0.04	0.04	0.2	0.1	0.08	0.04	0.2
Waubesa	0.5	0.2	0.06	0.5	0.8	0.6	0.3	0.9
Kegonsa	0.1	0.1	0.04	0.3	0.4	0.3	0.09	0.3

nutrients indicate there were differences in the growth of algae in water from Lakes Mendota and Monona when samples were collected during high flows *versus* low flows. Much higher growths were achieved in the samples from the high-flow period. This is a logical response since growth in these lake waters was more limited by N or Fe, and surface runoffs apparently supply these nutrients during the winter in much the same manner as in the summer. The fact that there was little difference in the growth of the algae between high- and low-flow samples after they had been spiked with a combination of N and Fe indicates that surface runoffs at this time of year do not add significant quantities of P, which is the limiting factor when N and Fe are both added. There was little response to added N and Fe in the Lakes Wingra and Kegonsa samples because during the winter these lakes have very low levels of P. This is an interesting condition in the case of Lake Kegonsa because the flow from Lake Waubesa contains relatively high amounts of available P and additional P comes from the smaller inlets to this lake, Halverson and Door Creeks.

The relationship between the soluble PO_4-P and available P of these lake waters calculated from the data of samples spiked with a combination of N and Fe for both membrane-filtered and autoclaved samples is summarized in Figure 14. It is evident that in these lake waters, P analyzed as soluble PO_4-P is available to all three algae.

The data presented from growth tests of algae in lake waters have demonstrated: how the selected algae can be used to compare the fertility of different lakes; how the lakes will respond to additions of N, P, and Fe; the effect of high- *versus* low-flow periods during the winter on the available amounts of N and Fe in some of the lakes which are limited by the N and Fe levels; and the lack of effect of surface runoffs during winter in lakes which are limited by P.

The Soluble PO_4-P of Lake Waters and P Content of *In Situ* Algae

The results of analyses of the soluble PO_4-P of the lake water and the extractable PO_4-P of the *in situ* algae are presented in Figures 15-18. Rainfall data are also presented in Figure 1 Fall and winter (1972-1973) analyses of soluble PO_4-P are presented in Figure 19. The early *in situ* algae analyzed were *Ulothrix* sp and *Spirogyra* sp with *Cladophora* sp appearing in late May and continuing through September.

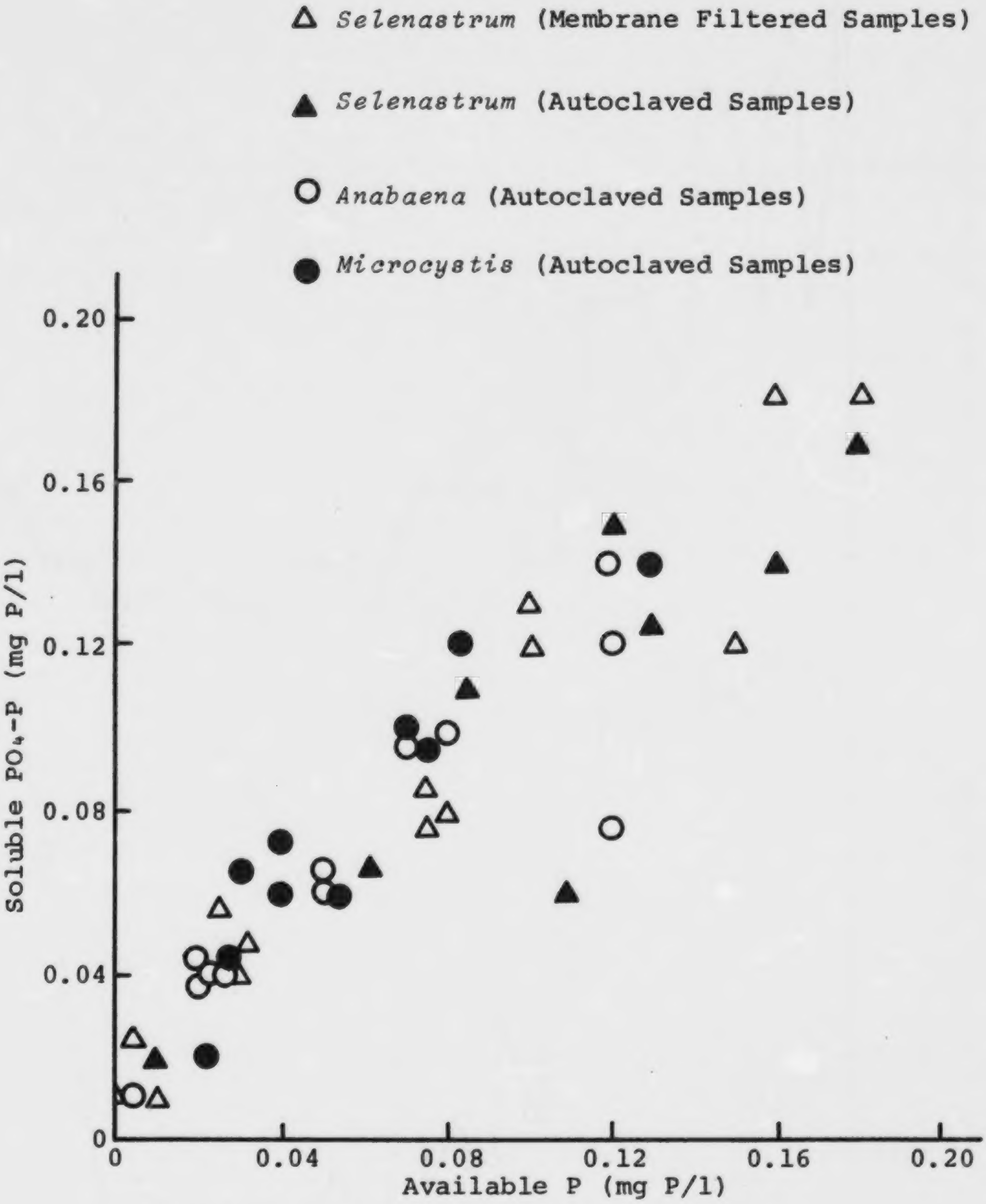

Fig. 14. Relationship Between Soluble PO_4-P of Lake Waters and Available P - Growth Tests January and February, 1973

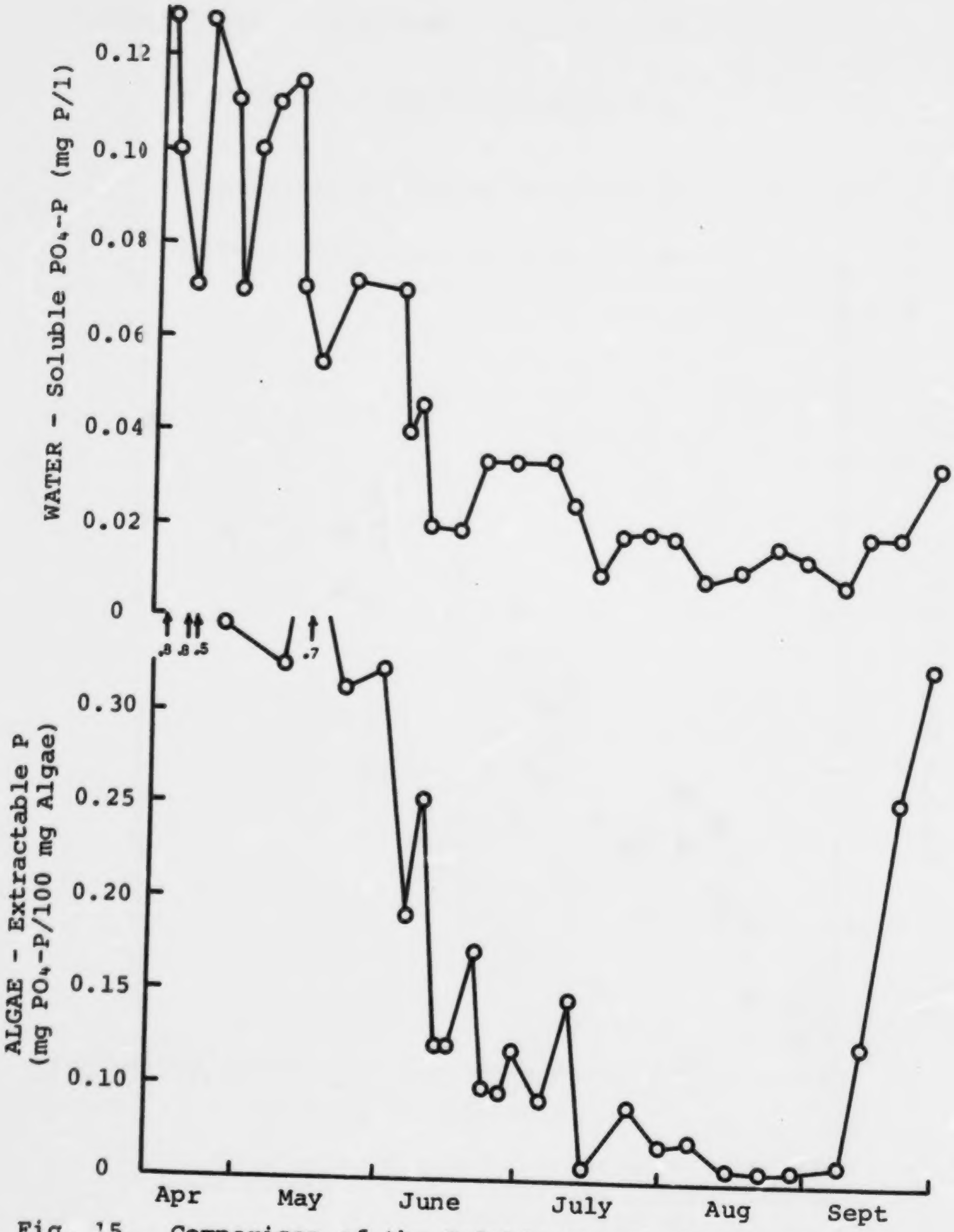

Fig. 15. Comparison of the Soluble PO_4-P of Lake Mendota Waters and the Extractable PO_4-P of *in situ* Algae at the Outlet

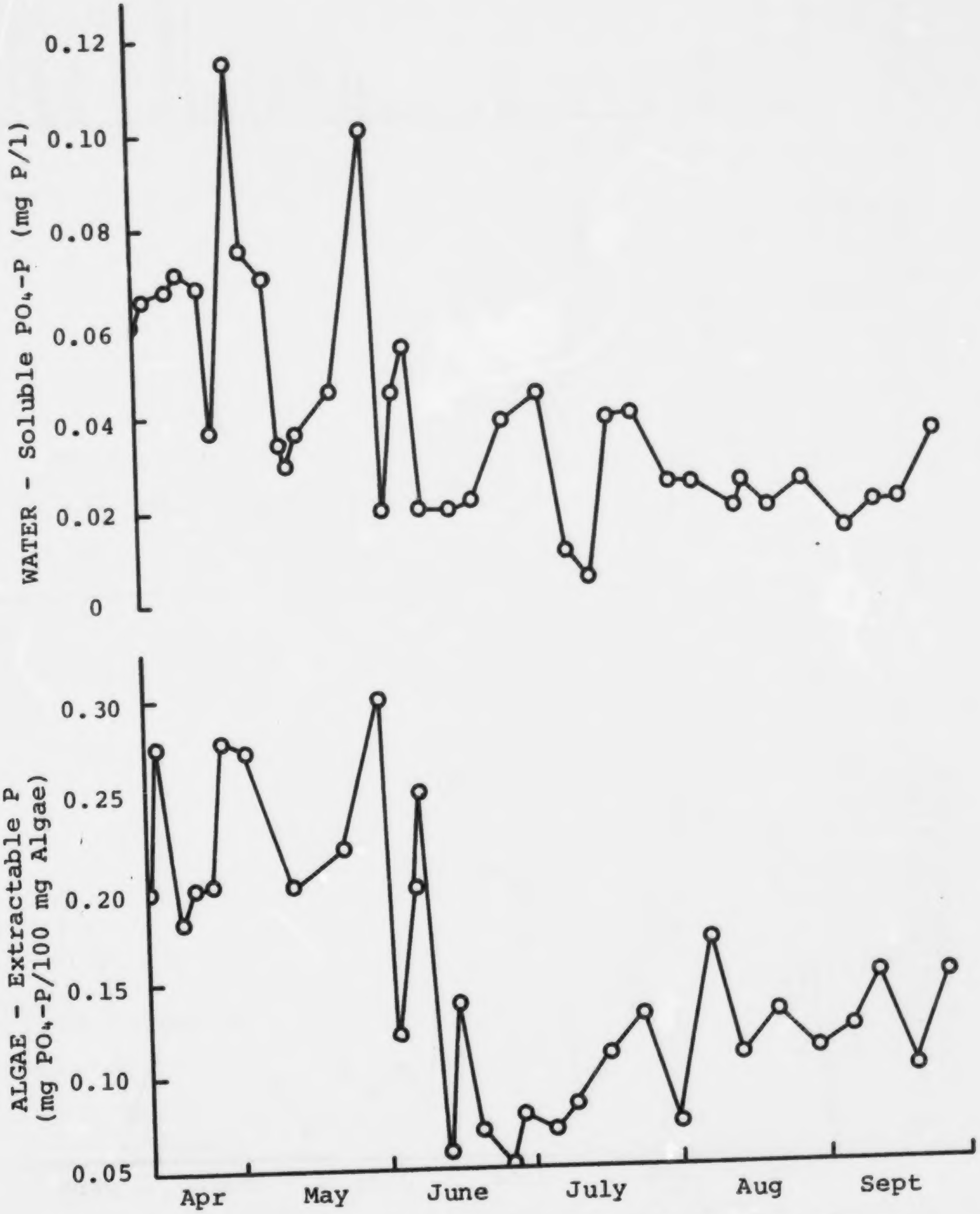

Fig. 16. Comparison of the Soluble PO_4-P of Lake Monona Waters and the Extractable PO_4-P of *in situ* Algae at the Outlet

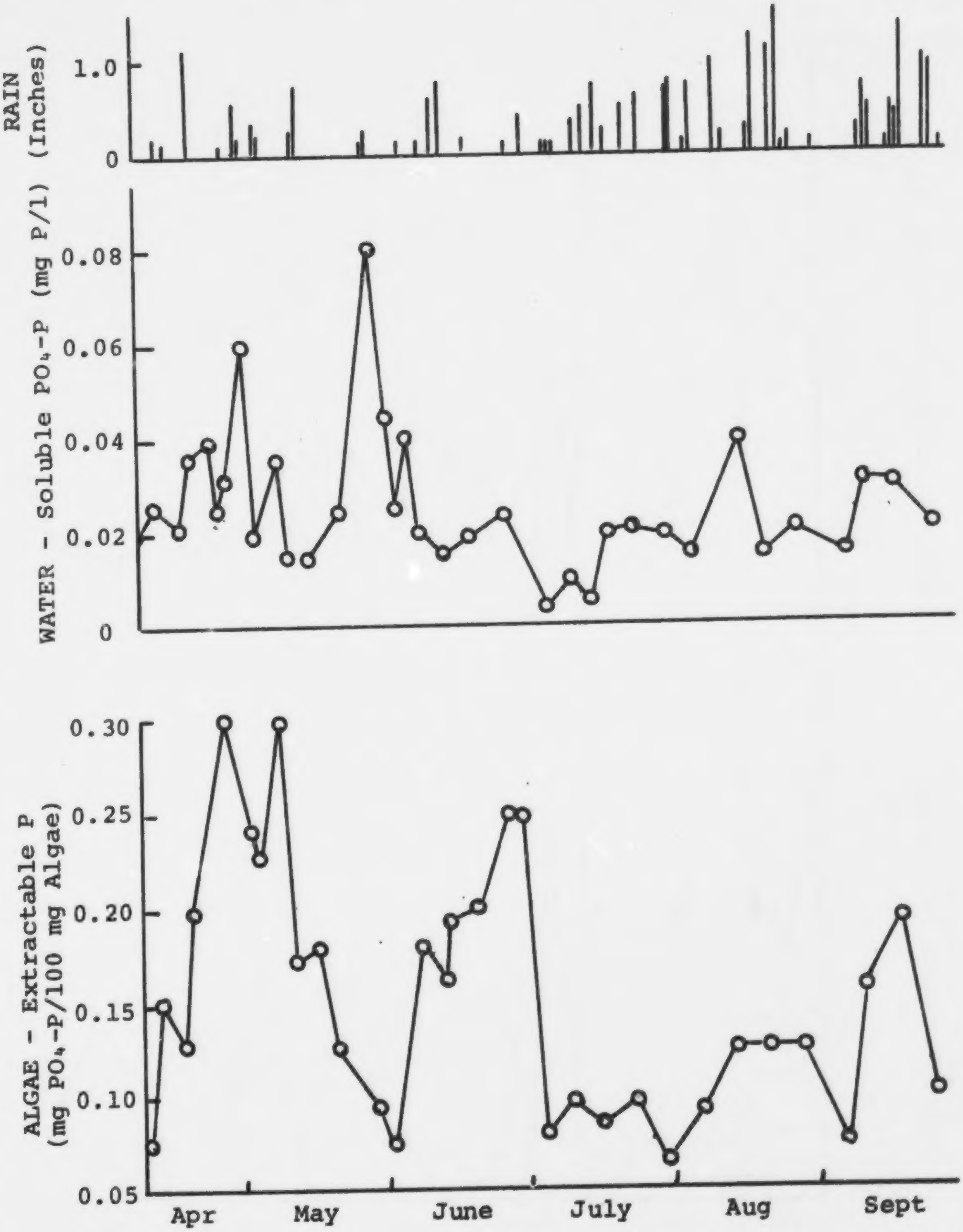

Fig. 17. Comparison of the Soluble PO_4-P of Lake Waubesa Waters and the Extractable PO_4-P of *in situ* Algae at the Outlet

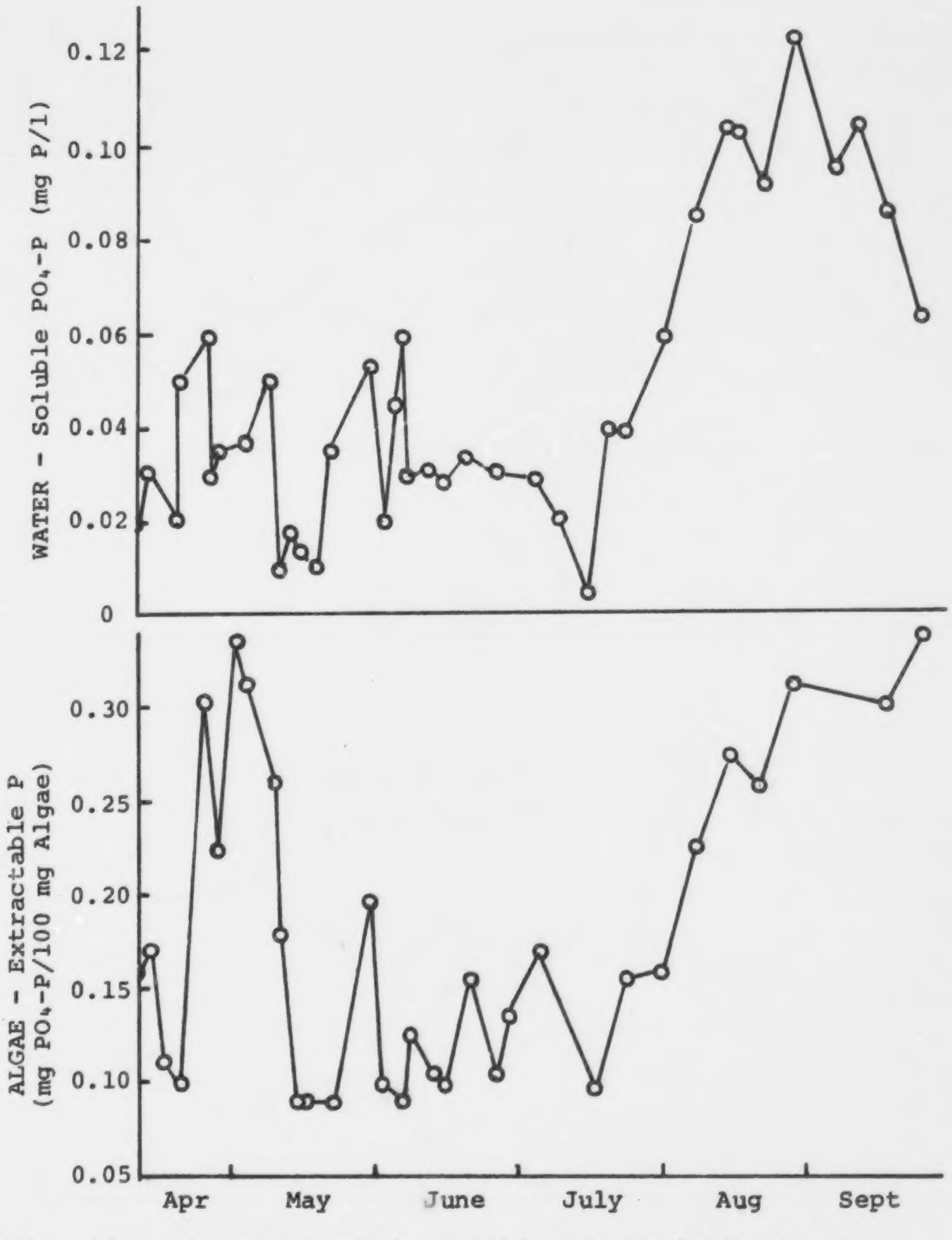

Fig. 18. Comparison of the Soluble PO_4-P of Lake Kegonsa Waters and the Extractable PO_4-P of *in situ* Algae at the Outlet

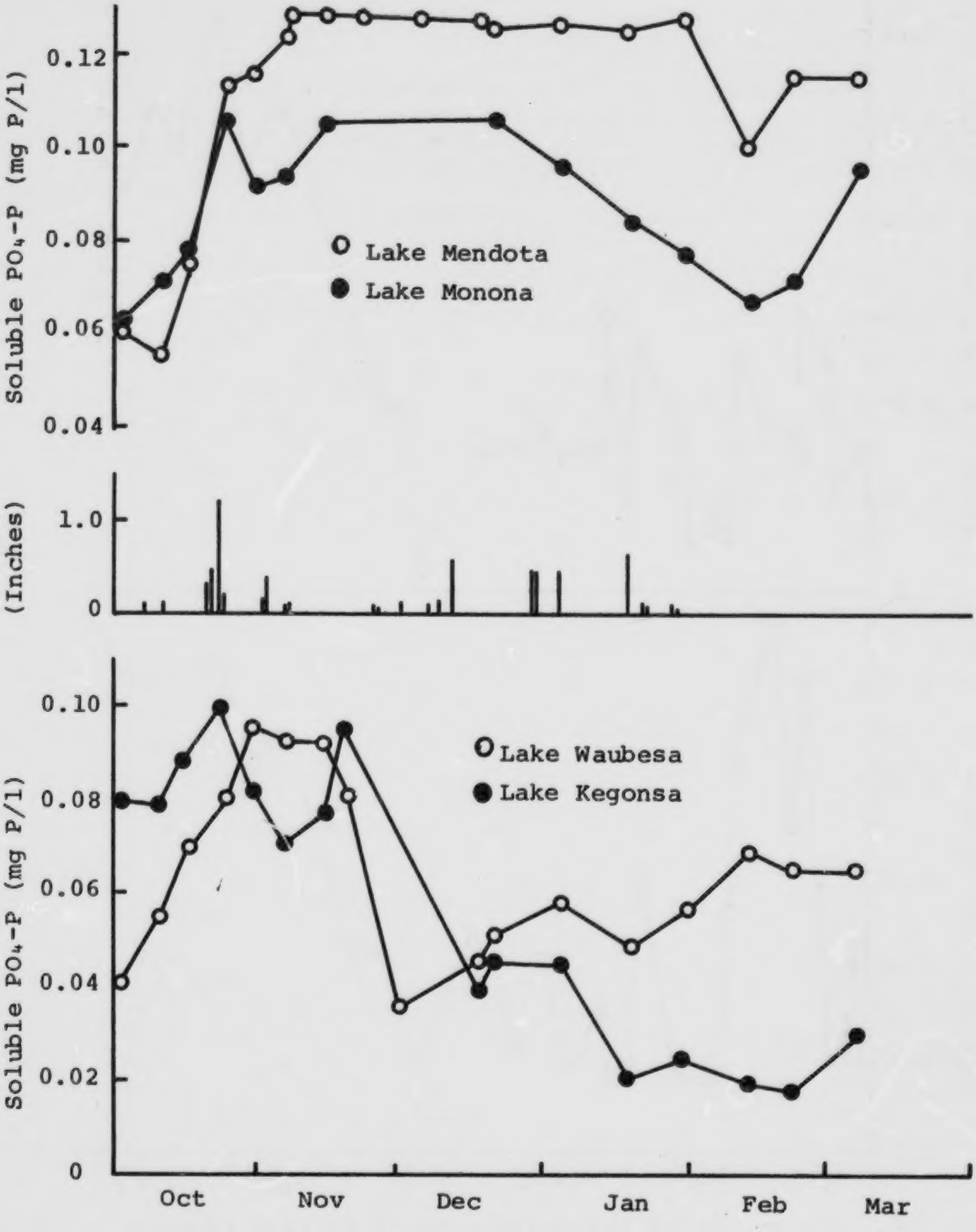

Fig. 19. Comparison of the Soluble PO_4-P of Madison Area Lakes, Fall and Winter, 1972-1973

During the sampling period of 1972 when the lakes were covered with ice, April 10 to 18, the soluble PO_4-P concentrations in Lake Mendota were around 0.1 mg P/l, and the extractable PO_4-P of the *in situ* algae in the lake outlet was very high, in the range of 0.5 to 0.8% P. The concentration of soluble PO_4-P in Lake Monona was about 0.065 mg P/l and the algae had 0.2% P or higher. In contrast, the concentration of soluble PO_4-P in both Lakes Waubesa and Kegonsa was about 0.02 mg P/l, and the algae had extractable PO_4-P levels of about 0.15 to 0.17% P. The results of analyses on Lake Mendota during the ice period of 1972-73 were about the same as in April, 1972. There was a gradual decrease in the soluble PO_4-P of Lake Monona from about 0.1 mg P/l in December to 0.07 mg P/l in February. There was then an increase to about 0.1 mg P/l in March. In contrast to the results of April, 1972, during the ice period of 1972-73 the soluble PO_4-P of Lake Waubesa remained at about 0.05 to 0.07 mg P/l from December to March. From mid-January to March, the soluble PO_4-P of Lake Kegonsa was less than 0.03 mg P/l, values very similar to those in April, 1972. Also, the algae (*Ulothrix*) in the outlet of Lake Kegonsa in March, 1973 had only about one-half the extractable P of the *Ulothrix* of Lake Mendota at this time.

After the ice breakup in 1972 there was a gradual decrease in the soluble PO_4-P of Lake Mendota from about 0.1 mg P/l to about 0.03 mg P/l by mid-June. The extractable PO_4-P of the algae remained relatively high until a drastic decrease to 0.1% P during June. The algae (*Cladophora* sp) of Lake Mendota remained P-limited (less than 0.08% extractable P) through September 6. They contained surplus P at September 11 and afterwards, although the fall overturn was not detected by chemical analyses of lake water until September 25. This demonstrates that sources of P are detected by analyses of *in situ* algae before they cause an increase in the surplus P of the lake waters (measured as soluble PO_4-P left in the water after algae have removed what they could).

There was a similar decrease in the soluble PO_4-P of Lake Monona, from 0.07 mg P/l to about 0.02 mg P/l by mid-June. As in the case of Lake Mendota, the P of the algae in Lake Monona remained relatively high, 0.2 to 0.3% P, until mid-June when it dropped to below 0.1% P. The soluble PO_4-P of Lake Waubesa remained at about 0.02 to 0.03 mg P/l throughout the spring and summer of 1972. However, there was a definite peak in the per cent P of algae in late April and early May, perhaps associated with rains and surface runoffs at this time. Since the algae of Lake Monona already had relatively high levels of P during this period, this effect on the algae of Lake Waubesa was more spectacular. The decrease in the P content of the algae in late May was probably associated with the algal blooms occurring in all the lakes (mainly green algae and diatoms). There was another peak in the P content of the algae of Lake Waubesa in June, again probably associated with rains and surface runoffs. This increase in the P content of the *in situ* algae during a period of little change in the soluble PO_4-P of the water demonstrates the value of *in situ* algae in detecting sources of P that are not great enough to exhibit surplus P in the water. The large amounts of rain during July through September after the farm fields and marshes were green did not appear to affect the surplus P of the lake, measured as soluble PO_4-P, but the P content of the algae indicated that both Lakes Monona and Waubesa received enough available P during this period for the algae to have about 0.12% P at a time when the algae of Lake Mendota had less than 0.08% P. Since Mendota receives relatively little urban drainage compared to Lake Monona, the increased P in the algae of Lake Monona may be due to this source. However, the soluble PO_4-P of Lake Wingra, which receives over 90% of its P from urban drainage from the City of Madison, was always barely detectable and the *in situ* algae always P-limited. The only exceptions were when ducks congregated near the sampling stations on Lake Wingra and relatively higher P values were found in water and algae samples. The P of the Lake Waubesa algae is probably due to a combination of farm runoff and septic tank drainage from the areas around this lake that are not yet sewered.

The soluble PO_4-P of Lake Kegonsa increased after ice out and then decreased in mid-May. The algae also exhibited a peak in their P content during this period. The soluble PO_4-P of Lake Kegonsa increased to about 0.04 mg P/l in early June, but then fell to 0.02 to 0.03 mg P/l during late June and early July, much like the results in Lake Mendota. Despite a series of rains (July 12 to 19) which amounted to about 1.8 inches, the P content of Lake Kegonsa and its algae did not increase. Therefore, surface runoffs do not appear to be a major source of P to this lake at this time. There was a spectacular increase in the soluble PO_4-P of Lake Kegonsa, to about 0.1 mg P/l, during August through September. The P content of the algae also increased during this period. Since the P in all of the other Madison lakes did not increase during July and August, the increase in the P of Lake Kegonsa is due to local factors. The lack of an increase in the soluble PO_4-P of Lake Waubesa might be used to indicate that surface runoffs to these two lakes during August (4 days had rains in excess of 1 inch) did not cause the great increase in the P of Lake Kegonsa. Therefore, the high levels of P in Lake Kegonsa most probably are due to the septic tank drainage from the summer population around this lake as compared to the effects of the summer population around Lake Waubesa which was in the process of being sewered. If there is a spectacular decrease in the soluble PO_4-P and P content of the *in situ* algae in Lake Kegonsa when the proposed sewers replace the use of septic tanks around this lake, the comparative effect of surface runoffs from very fertile farm lands, which will continue, and the septic tank drainage can be conclusively demonstrated.

Lake Kegonsa demonstrates a tremendous capacity to assimilate the soluble P added from Lake Waubesa and its smaller tributaries, Halverson and Door Creeks, during the winter so that relatively little soluble PO_4-P leaves Lake Kegonsa, and the algae at the outlet have comparatively low P values. This situation also took place during the winter of 1943-44 when Lakes Waubesa and Kegonsa were receiving the effluent of the Madison Sewage Treatment Plant.[5] At that time, despite the fact that the water entering Lake Kegonsa had 0.3 to 0.5 mg PO_4-P/l (December, 1943 through April, 1944), there was 28% less P leaving the lake as either soluble PO_4-P or total P. In contrast, during the recent summer, when algae and aquatic plants are considered to be very effective in removing nutrients from lake waters, the soluble PO_4-P and the P content of the algae of this lake increased. During the summers of 1943 and 1944, the outlet of Lake Kegonsa contained as much soluble PO_4-P and total P as the inlet. Therefore, the sources of P to Lake Kegonsa during the summer must be tremendously more important than winter sources. The fact that the low-lying cottages surrounding Lake Kegonsa are used mainly only during the summer, as compared to the more permanent homes on relatively higher grounds, tends to indicate that the cottages on lower grounds may be the source of the excess P to this lake during the summer. If this is the case, which will be tested when the cottages are sewered, then the effects of this source of P, which appear to not continue past November, and the sewering of this population should have a measurable effect on the lake during the first year.

Sources of Phosphorus to the Madison Area Lakes

The value of bioassays for available P as compared to chemical analyses for soluble PO_4-P or total P is clearly demonstrated by results of tests of some of the tributaries to the Madison lakes.

Lake Mendota - University Bay Creek is essentially a storm sewer serving a portion of the west side of the City of Madison. In addition, it receives some cooling waters from the University of Wisconsin campus which insure a continuous but minor flow. In 1970, samples of water from this creek were tested and found to have spectacular amounts of available P, effects of this P were noted in the creek and at 25 and 250 meters from shore by using P-limited *Anabaena* and N_2-fixation tests.[6] In 1971, chemical analyses indicated approximately 0.1 mg P/l was present in two samples of creek water, but tests for the availability of the P were negative because the water samples were toxic to P-limited *Anabaena.* It was at this time that biocides were being used in some of the University of Wisconsin cooling towers. In 1972, 8 tests were carried out on University Bay Creek waters during periods of low and high flow (rain). The soluble PO_4-P ranged from 0.025 to 0.12 mg P/l. In 6 tests no available P was detected by sorption tests, and in the other two tests less than 1/3 of the soluble PO_4-P present was available. However, when creek waters were autoclaved (6 tests) or allowed to age in the laboratory for 8 days (6 tests), the soluble PO_4-P of the treated creek waters was available in both sorption tests with *Cladophora* and growth tests with *Selenastrum.* Autoclaving these samples did not change the concentration of soluble PO_4-P so the reason for decreased toxicity is not yet known.

The Yahara River and Token Creek are tributaries entering Lake Mendota from the north and represent the main source of water. Both have flows during dry periods. Upstream samples collected from the Yahara River near the Town of Windsor during the period of August through December, 1972 indicated that, although there were 0.10 to 0.15 mg soluble PO_4-P/l present, there was no available P in 6 sorption tests and less than 1/3 of the PO_4-P was available in 3 tests. No increase in availability could be detected in membrane-filtered samples or by the addition of EDTA (7 mg/l). Autoclaved samples containing 0.14 mg PO_4-P/l were also found to contain no available P when tested in growth tests with *Selenastrum.* Upstream samples of Token Creek water (Wis Highway 19) were tested 6 times, and in 4 tests less than 30% and in 2 tests only 50% of the soluble PO_4-P was available by sorption tests. In contrast, 22 tests with downstream samples of the Yahara River water (Wis Highways 19 and 113) indicated that the soluble PO_4-P was available either as raw samples in sorption tests or as autoclaved samples in growth tests with *Selenastrum*. Thus, there appears to be a difference in the availability of compounds analyzed as soluble PO_4-P in these rivers or creeks. Downstream samples appear to have nearly the same chemical analyses for soluble PO_4-P, but the P present is readily available to algae, in contrast to the P of upstream samples.

Lake Monona - The main source of water to Lake Monona is the Yahara River coming from Lake Mendota. We have tested two tributary sources of P to the Yahara River below Lake Mendota, the Tenney Park Lagoon and the Johnson Street storm sewer. The lagoon is a shallow lake, and from July through mid-August, 1972, it was filled with aquatic weeds (mainly *Myriophyllum* sp). The lagoon also has a very heavy population of ducks. In mid-August the majority of the weeds were cut by mechanical harvesters and removed from the area. Preliminary tests indicated that more soluble PO_4-P was coming out of the lagoon than entered, so a series of analyses were made of the water and *in situ* algae in the lagoon area to evaluate the lagoon as a source of P to the Yahara River and Lake Monona, to detect any changes in the lagoon after the weed removal operation, and to measure any changes after the fall overturn of Lake Mendota. The data collected are summarized in Table 31. On three different dates in early August, it was found that the water coming from the lagoon contained significantly higher concentrations of soluble PO_4-P and available P, and the algae at the outlet and close below contained higher amounts of extractable PO_4-P (i.e., data of 8/4/72). Samples collected soon after the weed removal operation indicated that the lagoon outlet waters still had more P than inlet waters (data of 8/21/72), but no more than before the weeds were removed. Thus, the weed cutting operation, which consisted of cutting, collecting, and roller-squeezing to remove entrained surface moisture before removal from the lagoon area, did not cause a measurable increase in the P content of the outlet water of the lagoon. Tests in mid-September also indicated that surplus P was coming from the lagoon system. It was therefore concluded that the P released from the lagoon was not related to the presence, removal process, or absence of the dense growths of water milfoil. After the turnover of Lake Mendota, near early October, the results of further tests in the lagoon area indicated that the lagoon outlet contained

Table 31. COMPARATIVE ANALYSES OF THE YAHARA RIVER AND TENNEY PARK LAGOON AT DIFFERENT TIMES

Date and sample area	Soluble PO_4-P (mg P/l)	Available P (sorption) (mg P/l)	*In situ* algal P (extractable PO_4-P) (% P)
8/4/72			
Mendota outlet	0.02	0.02	0.06
Lagoon inlet	0.01	0.02	0.07
Above Lagoon outlet	0.02	-	0.07
Lagoon outlet	0.04	0.04	0.20
Below Lagoon outlet, 50 M	0.02	-	0.10
Below Lagoon outlet, 300 M	0.02	0.02	0.06
8/21/72			
Mendota outlet	0.018	-	0.05
Lagoon outlet	0.044	-	0.11
9/14/72			
Mendota outlet	0.016	-	-
Lagoon inlet	0.018	-	0.072
Lagoon outlet	0.030	-	0.10
Below Lagoon outlet, 300 M	0.022	-	0.072
11/8/72			
Mendota outlet	0.12	0.14	0.30
Lagoon inlet	0.12	0.12	0.26
Above Lagoon outlet	0.12	0.14	-
Lagoon outlet	0.06	0.04	0.17
Below Lagoon outlet, 300 M	0.12	0.08	0.39

less soluble PO_4-P than the now relatively fertile water in the Yahara River. The *in situ* algae also indicated that there was little change in the amount of P available at the lagoon outlet, whereas the P content of the algae in the Yahara River had increased at least fourfold (data of 11/8/72). Thus, during the summer the lagoon systems can be looked on as being a minor source of available P to the Yahara River, but not to Lake Monona; it had a minor diluting effect during high nutrient periods after fall overturn.

Concern for the potential fertilization of the Yahara River, and eventually Lake Monona, by the water of the Johnson Street storm sewer caused a number of analyses to be made of the sewer as a source of available P. This sewer receives cooling water discharges from industries and has a relatively high flow of warm water the year round. As has been mentioned, during August and early September, the water leaving Lake Mendota was nearly devoid of soluble PO_4-P and the algae at the outlet were P-limited. Tests between Lakes Mendota and Monona indicated that there was soluble PO_4-P entering the river from the sewer (as high as 0.1 mg P/l) and the concentration of soluble PO_4-P below the sewer was slightly elevated (0.07 mg P/l). The algae collected in this part of the river were *Cladophora* sp which were so heavily coated with epiphytic diatoms that they appeared brown to black. When the algae were separated it was found that the untreated mixture had 0.067% extracted P; the cleaned *Cladophora* had 0.075% P; and the diatoms had 0.025% P. Thus, although different, all the algae were P-limited. To test if the algae were alive and capable of absorbing P, if it had been available to them, 20 mg samples were incubated 20 hours in 400 ml samples of Lake Mendota water (0.01 mg PO_4-P/l) and Yahara River water from 200 meters below the storm sewer (0.066 mg PO_4-P/l), and samples of each source to which 0.04 mg PO_4-P had been added. The extractable PO_4-P of the *Cladophora* plus epiphytes after such incubations was 0.078% P for either water sample despite the difference in chemical analyses. However, samples containing the added PO_4-P had extractable PO_4-P levels of 0.09 to 0.10% P, indicating that, although these algae could not utilize some of the P present below the storm sewer, they were capable of absorbing PO_4-P added to the samples. Further sorption tests using Lake Mendota and Lake Wingra *Cladophora* sp and growth tests of autoclaved samples using *Selenastrum* were carried out to determine if the soluble PO_4-P of the Johnson Street storm sewer was available P. Tests (9/14/72) with Lake Wingra *Cladophora* indicated the extractable PO_4-P of the algæ increased from 0.06% P to 0.11% P when incubated in water samples containing 0.05 mg PO_4-P/1, whereas when incubated in sewer waters containing 0.10 mg PO_4-P/1 (undiluted) or 0.05 mg PO_4-P (diluted with P-free culture medium), the algae had extractable PO_4-P concentrations of only 0.06% P. Tests carried out (12/20/72) with autoclaved samples using *Selenastrum*, when Lake Mendota contained 0.15 mg PO_4-P/1 and the sewer water contained 0.057 mg PO_4-P, indicated that available P was present in the sewer water. When P was added to the sewer waters, the growth of *Selenastrum* was significantly higher than when tested "as is" or with the addition of N plus Fe. Thus, the lack of availability of the storm sewer P was not due to only toxic factors.

The results of these tests indicate that: 1) the Tenney Park Lagoon system adds available P to the Yahara River during the summer but dilutes the level of P in the river after fall overturn; 2) the source of the higher levels of P in the Tenney Lagoon could not be related to the presence of weeds or a new type of cutting operation carried out; 3) the Johnson Street storm sewer contributes compounds to the Yahara River which analyze as soluble PO_4-P but are not available for *in situ* algae or test algae.

Lake Kegonsa - The main tributary to Lake Kegonsa is the Yahara River coming from Lake Waubesa. Tests carried out on this section of the river indicated that there was little change in the chemistry of the water or availability of P from the Lake Waubesa outlet to the Lake Kegonsa inlet. Two smaller tributaries, which are believed to have less than 10% of the flow from Lake Waubesa, are Halverson and Door Creeks. These small creeks have a continuous flow, pass through relatively fertile farm lands, but are unmodified by passage through widespread areas. Bioassays for available P were carried out with these creek waters on 26 dates from August, 1972 to March, 1973. A summary of results is presented in Table 32 as a comparison of analyses for total P and soluble PO_4-P with available P calculated from 1-day sorption tests or growth tests using *Selenastrum*.

It is evident from the data that the available P of the lake water samples is more closely related to the soluble PO_4-P than total P, as has been pointed out previously.[4] In contrast to the soluble PO_4-P of lake waters, the soluble PO_4-P of the creek waters was sometimes not available. There appears to be a difference in the availability of the P from surface runoffs

Table 32. COMPARISON OF CHEMICAL ANALYSES OF PHOSPHORUS AND AVAILABLE PHOSPHORUS IN LAKE WATERS AND CREEKS

	Chemical analyses		Available P	
Date and sample area	Total P (mg P/1)	Soluble PO_4-P (mg P.1)	Sorption (mg P/1)	Growth (mg P/1)
9/25/72[a]				
L. Waubesa	0.080	0.034	0.028	-
L. Kegonsa	0.13	0.064	0.080	-
Halverson Cr	0.16	0.11	0.060	-
Door Cr	0.14	0.088	0.050	-
11/15/72				
L. Mendota	-	0.15	0.18	0.07
L. Monona	-	0.11	0.085	0.07
L. Waubesa	-	0.092	0.045	0.06
L. Kegonsa	-	0.078	0.028	0.05
Halverson Cr	-	0.13	0.035	0.032
Door Cr	-	0.10	0.	0.026
11/27/72				
L. Mendota	-	0.13	0.10	-
L. Waubesa	0.066	0.034	0.030	-
Halverson Cr	0.19	0.026	0.	-
Door Cr	0.20	0.056	0.	-
12/18/72[a]				
L. Mendota	0.14	0.14	0.12	0.12
L. Waubesa	0.073	0.046	0.053	0.068
L. Kegonsa	0.080	0.050	0.055	0.058
Halverson Cr	0.17	0.060	0.	0.005
1/18/73[a]				
L. Mendota	-	0.13	0.13	0.16
L. Monona	-	0.080	0.11	0.11
L. Waubesa	-	0.046	0.06	0.062
L. Kegonsa	-	0.02	0.02	0.02
Halverson Cr	-	0.20	0.19	0.18
Door Cr	-	0.38	0.36	0.31
3/15/73[a]				
L. Mendota	0.19	0.10	0.12	-
L. Monona	0.14	0.10	0.10	-
L. Waubesa	0.17	0.12	0.14	-
L. Kegonsa	0.15	0.044	0.05	-
Halverson Cr	0.16	0.082	0.	-
Door Cr	0.27	0.14	0.12	-

[a] High flow periods

and ground waters. During periods of high flow, after rains or snow melts when the creeks contained surface runoffs, the soluble PO_4-P was generally available: in 30 water tests, 26 had available P.

During low flow periods when creek flow was mainly ground water, the available P of 30 water tests was below 30% of the soluble PO_4-P and the majority of the tests had no available P. Detailed tests have not determined whether the unavailability of the ground water P was due to interference in PO_4-P analyses by arsenic, but occasional increases in availability after periods of room temperature storage or autoclaving have been noted, so at this time final conclusions can not be made.

References

1. Lawton, G. W. Limitation of Nutrients as a Step in Ecological Control. In: Algae and Metropolitan Wastes. Cincinnati, Taft Sanitary Engineering Center, Technical Report W61-3, U.S. Public Health Service, 1961. p. 108-117.
2. Fitzgerald, G. P., and G. F. Lee. Use of Tests for Limiting or Surplus Nutrients to Evaluate Sources of Nitrogen and Phosphorus for Algae and Aquatic Weeds. Madison, Water Chemistry Program Report, University of Wisconsin-Madison, 1970. 31 p.
3. Fitzgerald, G. P. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters, Allen, H. E., and J. R. Kramer (ed.). New York, John Wiley and Sons, 1972. p. 147-170.
4. Fitzgerald, G. P., S. L. Faust, and C. R. Nadler. Correlations to Evaluate Effects of Wastewater Phosphorus on Receiving Waters. Water and Sewage Works. 120(1): 48-55. 1973.
5. Sawyer, C. N., J. B. Lackey, and A. T. Lenz. Investigation of the Odor Nuisance Occurring in the Madison Lakes. Madison, Wis., Governor's Committee Report. 1944. 137 p.
6. Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burris. Acetylene Reduction Assay for Determination of Phosphorus Availability in Wisconsin Lakes. Proc. National Acad. Sci. 66:1104-1111. 1970.

SECTION VIII

LAKE RESTORATION EFFECTS AS MEASURED BY ALGAL ASSAY: BOTTLE TEST

Introduction

The overall objectives of this aspect of the project were: 1) to measure changes in the nutrient status of lakes brought about by lake restoration procedures, and 2) to evaluate the usefulness of the Algal Assay Procedure: Bottle Test as a predictive and interpretive tool for lake management.

This project was conducted in conjunction with the Inland Lake Renewal and Management Demonstration Program, a cooperative project involving the University of Wisconsin-Extension and the Wisconsin Department of Natural Resources. As part of that program, several Wisconsin lakes were manipulated for improved water quality. Restoration techniques included dilutional pumping, nutrient inactivation/precipitation with aluminum (alum or sodium aluminate) and hypolimnetic aeration without destratification. The lakes under study were monitored for a wide spectrum of physical, chemical and biological parameters. This project expanded the data collection program by the addition of algal assay results and, at the same time, provided data for evaluation of algal assay results against a backdrop of diverse limnological parameters.

The experimental design of the bioassays was based solely on maximum cell production; exponential growth rates were not measured. Each assay was designed to yield the following information for a particular sample: 1) the algal biomass produced by the sample (autoclaved), 2) the nutrient which limited cell production, 3) the quantity of biologically available phosphorus in the sample, and 4) the quantity of

Table 33. ALGAL BIOASSAYS CONDUCTED

Lake name	Treatment and date	No. assays[a] prior to treatment	No. assays[a] after treatment
Horseshoe	nutrient inactivation with aluminum (5/70)	-	16
Snake	nutrient inactivation with aluminum (5/72)	10	19
Long	nutrient inactivation with aluminum (5/72)	5	16
Pickerel	nutrient inactivation with aluminum (4/73)	25	6
Mayflower	not treated	5	0
Mirror	hypolimnetic aeration (1/73)	21	7
Larson	hypolimnetic aeration (2/73)	16	18
Horsehead	drawdown/winter aeration (?)	5	0
Windfall	aquatic plant mgmt	5	
Shadow	NA	6	
White	winter aeration	1	

[a] Each assay consists of 2 samples, one from the epilimnion and one from the hypolimnion.

biologically available nitrogen in the sample. In addition, checks were run for possible toxicity in the sample, and a series of reference cultures were incubated along with the lake samples to establish the sensitivity of the inoculum to increasing levels of N and P. It is emphasized that the algal assay results alone are not sufficient to evaluate the efficacy of the lake renewal techniques under study. The purpose of this study was to evaluate the usefulness of the standardized Algal Assay Procedure: Bottle Test under the practical constraints of a demonstration program. A summary of the assays conducted as part of this program is given in Table 33.

Methods and Procedures

Sample preparation -

Upon receipt, normally on the day after collection, the sample waters were autoclaved at 15 psi for 15-30 minutes, cooled, sealed and stored at room temperature until tested. Precipitates which form as a result of autoclaving were redissolved by bubbling CO_2 through the sample. The pH was then adjusted to 7.5 ± 0.3 by aeration.

Chemical analysis -

Complete chemical analyses of the raw samples were conducted by the Wisconsin Department of Natural Resources. In addition, because in certain waters the phosphorus concentration is altered upon autoclaving, each autoclaved sample was analyzed for soluble PO_4-P by the stannous chloride method (Standard Methods). Thus, the phosphorus concentrations obtained from the bioassays were comparable to those obtained from chemical analyses.

Inoculum -

Algal species - The bioassay organism used throughout the study was *Selenastrum capricornutum.* Stock cultures were maintained in triple-strength, autoclaved nutrient medium. The age of the culture used for inoculation purposes ranged from 7 to 21 days.

Inoculum preparation - The inoculum was prepared by centrifuging a portion of a 7- to 21-day-old culture of *Selenastrum capricornutum*, discarding the supernatant, and resuspending the cells in a solution of $NaHCO_3$ (45 mg/l in glass-distilled water) to give a final concentration of 1000 cells/ml when added to the sample flasks.

Synthetic medium -

The composition of the synthetic nutrient medium is as follows:

Macronutrients

The following salts (biological or reagent grade) in milligrams per liter of glass-distilled water:

Compound	Concentration (mg/l)	Element	Concentration (mg/l)
$NaNO_3$	25.5	N	4.2
K_2HPO_4	1.0	P	0.186
$MgCl_2$	5.7	Mg	2.9
$MgSO_4 \cdot 7H_2O$	14.7	S	1.9
$CaCl_2 \cdot 2H_2O$	4.4	C	2.1
$NaHCO_3$	15.0	Ca	1.2
		Na	11.0
		K	0.469

Micronutrients

The following salts (biological or reagent grade) in micrograms per liter of glass-distilled water:

Compound	Concentration (μg/l)	Element	Concentration (μg/l)
H_3BO_3	185.	B	32.460
$MnCl_2$	264.	Mn	115.3
$ZnCl_2$	32.7	Zn	15.6
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.87
$FeCl_3$	96.0	Fe	33.0
$Na_2EDTA \cdot 2H_2O$	300.0		

The medium was used (autoclaved) in triple strength for the stock cultures, and (made up either without phosphorus or without nitrogen) in normal strength for the reference cultures. There was no autoclaving or filtering of the reference medium.

Assay procedure -

Lake waters were treated according to the following protocol:

Treatment	Concentration (mg/l) of added nutrient	Replicates
A Lake water control	0	3
B " " + P	0.05P	3
C " " + N	0.5N	3
D " " + P + N	0.05P, 0.5N	3
E " " + P + N + Fe	0.05P, 0.5N, 0.03Fe	3
F " " + P + Fe	0.1P, 0.03Fe	3
G " " + N + Fe	2.0N, 0.03F	3

Treatment A provided a measure of sample fertility and was used as a base of comparison for the other treatments.

Treatments B and C provided the data for determination of the limiting nutrient of the sample by comparing this response to that of the control.

Treatment D was included to check the possibility that both nitrogen and phosphorus were in relatively short supply, and additions of either of the nutrients added singly would not yield a measurable response.

Treatment E was designed to test for possible sample toxicity. Since algal growth is usually limited by only nitrogen, phosphorus, or possibly iron, unless toxic, virtually all waters, no matter how infertile, should produce measurable growth with this spike.

Treatments F and G were designed to force the sample into a situation where either phosphorus or nitrogen was limiting, and biologically available concentrations were determined by comparison with the proper reference curve.

In order to determine the nutrient content of the water samples, the response of the test alga to known levels of the nutrient in question must be measured. Therefore, the following set of reference spikes was included with each test:

1 medium (-) P[a]
2 " (-) P + 0.025 mg/l P
3 " (-) P + 0.05 mg/l P
4 " (-) P + 0.075 mg/l P
5 " (-) P + 0.186 mg/l P
6 medium (-) N[b]
7 " (-) N + 0.25 mg/l N
8 " (-) N + 1.0 mg/l N
9 " (-) N + 4.2 mg/l N

[a] Synthetic nutrient medium made without phosphorus.
[b] Synthetic nutrient medium made without nitrogen.

The phosphorus spike of all treatments was added as K_2HPO_4; the nitrogen spike as $NaNO_3$; and the iron as $FeCl_3$ + $Na_2EDTA \cdot 2H_2O$.

Because of spatial limitations, it was necessary to use sample volumes of 20 cc in 50 cc Erlenmeyer flasks resulting in surface to volume ratios within the range specified.

Incubation -

Each experimental setup was incubated at 24°C, with illumination of 400 ft C. Flasks were swirled daily.

Harvesting techniques -

Although many of the samples assayed were relatively fertile, the concentrations of cells produced were normally insufficient to permit direct gravimetric determination of algal biomass. Therefore, biomass was determined indirectly by measuring fluorescence of *in vivo* chlorophyll *a*. Two types of fluorometers were used, a Turner (III) and an Amnico unit.

Tests conducted during the initial stages of the project established that maximum fluorescent readings were obtained from cultures which had been incubated for 5-9 days and that readings often decreased significantly after day 9. As a result, a standard incubation period of 7 days was selected, and all cultures were harvested at that age.

Determination of dry weight -

Fluorescent values of *Selenastrum* cultures grown in NAAM medium containing varying concentrations of phosphorus were obtained for day 7 of incubation and related to the absorbance values at day 14 of incubation. The absorbance values were then converted to μg/l dry weight, resulting in a linear relationship between day-7 fluorescence and final dry weight. The average day-7 fluorescent relationship to day-14 dry weight is given by

$$X = 8.41\ F$$

where X = dry weight at day 14 in mg/l
F = fluorescent units at day 7

Determination of biologically available N and P -

Fluorometric readings of the reference cultures were plotted against the known concentrations of nutrient (N or P) in each spike. By comparing the readings of the N+Fe- or P+Fe-spiked lake samples to these curves, the concentration of either nutrient which is available for algal growth was determined.

Presentation of data -

The results of bioassays for each lake were plotted as a time sequence to show: 1) the amount of algal biomass produced in the samples of autoclaved lake water and the nutrient which limited biomass production, and 2) the concentration of biologically available phosphorus in the samples as determined by the growth assays.

Nutrient limitation was determined by amending the samples with phosphorus and nitrogen, singly and in combination. If a nutrient addition resulted in a significant increase in biomass as compared to the unamended samples, then that nutrient was identified as limiting. Limiting nutrients are denoted on the plots by the symbols P, N, or PN adjacent to the data points. The symbol PN is used to denote the condition in which the addition of neither nitrogen nor phosphorus alone yielded a significant increase in biomass, but a significent response was obtained by a combined addition of these nutrients.

This procedural definition of "limiting" provides information regarding the relative supply of one nutrient as compared to another, but is not indicative of the abundance or scarcity of a nutrient on an absolute scale. Thus, a shift from phosphorus-limitation to nitrogen-limitation could result from either an addition of phosphorus or a reduction in the supply of nitrogen.

Results and Discussion

Nutrient inactivation/precipitation -

Nutrient inactivation by chemical means has been proposed as one method by which excessive algal growth in lakes might be reduced. In particular, the use of aluminum to reduce in-lake concentrations of phosphorus appears to hold promise.

The effectiveness of aluminum as a precipitating agent for phosphorus has been demonstrated in laboratory studies and has been used in industrial and municipal treatment processes. Treatment with aluminum results in the formation of hydrous oxide and a phosphate precipitate of low solubility. The flocculant precipitate effectively reduces the phosphorus concentration (dissolved inorganic and particulates) by sorption, precipitation, and physical entrapment. Removal of other nutrients (as well as color and turbidity) is also accomplished during settling or flotation.

Bioassays were conducted on samples from four lakes which were treated with aluminum: Horseshoe, Snake, Long, and Pickerel Lakes. In general, chemicals were applied to the lakes by injection through a manifold at a depth of about 30 cm behind propeller-driven water craft. This provided good mixing and permitted *in situ* floc formation.

Commercial standard ground aluminum sulfate (alum) ($Al_2(SO_4)_3 \cdot 14\ H_2O$), liquid aluminum sulfate and sodium aluminate ($Na_2O \cdot Al_2O_3 \cdot 3\ H_2O$) were used as sources of aluminum III. The liquids were diluted before addition; the ground alum was added as a slurry without further dilution.

Descriptions of variations in lake treatments are listed below.

Table 34. LAKE TREATMENT WITH ALUMINUM

Lake	Chemical used	Concentration Al (mg/l)
Snake	liquid alum liq sodium aluminate	12 mg/l in 80% total lake volume
Long	liquid alum liq sodium aluminate	14 mg/l in surface 1 ft
Pickerel	liquid alum	7.3 mg/l in total lake volume
Horseshoe	granular alum	18 mg/l in surface 2 ft

Description of lakes treated with aluminum -

Snake Lake - Vilas/Oneida Counties - Snake Lake is a slightly acid, brown-water seepage lake of potentially high fertility. It has a surface area of 5 hectares and a volume of $1.1 \times 10^5 m^3$. The maximum depth is about 6 meters and the length of the shoreline is 1.2 km. The bottom sediment is muck overlying sand and peat. For 22 years the lake received discharge from the Village of Woodruff sewage treatment plant, resulting in the loss of the natural fish population and excessive nutrient enrichment. This nutrient source was eliminated in 1964, but the lake remained highly eutrophic even though nutrient influx was drastically reduced. As a means of renewal, this lake was subjected to dilutional pumping in October 1969 (pilot test) and July-August 1970 as part of the Inland Lake Renewal Program. During the latter pumping period approximately three lake volumes of water were pumped from the lake. The lake was then refilled by seepage from groundwater. Subsequent limnological evaluations showed that although nutrient concentrations in the lake were reduced, the levels remained sufficiently high that additional attempts for renewal were deemed desirable. Nutrient inactivation was attempted in May 1972, when the lake was treated with alum and sodium aluminate.

Horseshoe Lake - Manitowoc County - Horseshoe Lake is an alkaline, clear-water lake of medium to high fertility. It has a surface area of 9 hectares and a volume of about

$4.7 \times 10^5 m^3$. The maximum depth is 17 m and length of shoreline is 1.8 km. The bottom sediments are primarily muck and marl. Aquatic plant growth is lush around the shoreline. For an undetermined number of years the lake received effluents from a nearby dairy and cheese processing plant stimulating nuisance algal blooms in the mid-1960's. Horseshoe Lake was treated with alum in May 1970.

Long Lake - Langlade County - Long Lake is a slightly colored lake of medium fertility. It has a surface area of 27.5 ha and a maximum depth of 7.3 m. The lake is narrow with a sinuous shoreline configuration. This shape, along with the protection afforded by surrounding tall pines, encourages the stagnation of surface waters during the summer and may contribute to the occasional nuisance algal blooms that have been reported. Long Lake was treated with alum in May 1972.

Pickerel Lake - Portage County - Pickerel Lake is a small, clear-water lake of medium to high fertility. It has a surface area of 21 ha and a maximum depth of 4.6 m. The shoreline is fringed with rooted aquatics and algal production is high during the summer season. The lake has been subject to frequent winterkill during recent years. The source of nutrient input is unknown at this time as there is but one development on the lake, a church camp which does not undergo heavy use. The lake was treated with liquid alum on April 17, 1973.

Mayflower Lake - Marathon County (not treated) - (40 ha, 8 m maximum depth) Samples have been collected for bioassays as part of a program to establish baseline data for this lake. The lake has had problems with rooted aquatic vegetation and algae, and future alum treatment is planned.

Lab studies on lake water samples treated with alum -

An experiment was done to determine whether 1) aluminum treatment of lake-water samples in the laboratory would decrease the amount of phosphorus available for assimilation by AAP organisms, and 2) introducing an amount of phosphorus (as PO_4) equivalent to that which was removed would stimulate growth at original levels. Samples from Snake Lake (Vilas-Oneida Counties) and Mirror Lake (Waupaca County) were used, representing soft and hard water lakes respectively.

Snake Lake - Several liters of autoclaved lake water (February, 1972) were treated with 10 mg Al/l using commercial liquid alum, and the pH was raised back to 7.5 by adding NaOH. The flocculant precipitate was settled for several days before the supernatant liquid was decanted for analysis. Reactive-phosphorus was determined spectrophotometrically (molybdate-stannous chloride) on raw, autoclaved, supernatant, and supernatant plus added phosphate waters. Biologically available phosphorus was determined by triplicate AAP analysis on the autoclaved, treated, treated-reconstituted, and standard media waters. In conjunction with the AAP analyses, NO_3, PO_4, and Fe^{+++} spikes were added to determine whether algal growth would be stimulated by these nutrients. A stimulatory response to a phosphate spike was interpreted as phosphorus limitation in the algal culture. Algal growth was measured fluorometrically, and was related to dry weight of organisms.

Table 35 shows that about 83% of the reactive phosphorus was removed by Al(III) treatment and subsequent settling of the floc. Biologically available P appeared to be reduced by an even greater amount. While severe toxicity was not a problem (moderate growth in supernate + phosphate), there was a reduction in the growth rate. The Mirror Lake data show this effect also. Table 36 shows the fluorometric response of cultures. The data show a substantial growth potential for the autoclaved lake water (89.6 mg dry weight of organism per liter) and algal growth was stimulated by nitrogen additions. Aluminum treatment resulted in a large decrease in

Table 35. PHOSPHORUS ANALYSES ON SNAKE LAKE WATERS IN mg/l

	Reactive P (spectro-photometric)	Biologically Available P (AAP)
Raw water	0.094	-
Autoclaved water	0.118	0.12
Supernate of treated water	0.020	0.002
Supernate + phosphate	0.110	0.058

Table 36. GROWTH RESPONSE OF AAP ORGANISMS TO NUTRIENT REMOVAL AND SPIKES

Added nutrients in mg/l	Fluorometric response units[a] (mean and range)	Equivalent mg/l dry weight of organisms
AAP medium (-) P	21 (15-27)	-
" " (-) P + 0.025 P	1000 (980-1000)[b]	-
" " (-) P + 0.05 P	2300 (2200-2600)	-
" " (-) P + 0.075 P	3700 (3600-3900)	-
AAP medium (-) N	9 (7-10)	-
" " (-) N + 0.25 N	600 (580-620)	-
" " (-) N + 1.0 N	2700 (2600-2800)	-
Autoclaved lake water	2800 (2400-3200)[c]	90.
+ 0.05 P	2400 (2400-2500)	-
+ 0.5 N	4600 (4200-5200)	-
+ 0.05 P + 0.5 N	3600 (3000-4000)	-
+ 0.05 P + 0.5 N + 0.03 Fe	3800 (3700-3800)	-
+ 0.1 P + 0.03 Fe	2200 (2000-2300)	-
+ 0.2 N + 0.03 Fe	6000 (5700-6200)	-
Supernate of treated water	22 (20-25)[c]	0.70
+ 0.05 P	1500 (940-2100)	-
+ 0.5 N	25 (23-27)	-
+ 0.05 P + 0.5 N	1600 (1300-1800)	-
+ 0.05 P + 0.5 N + 0.03 Fe	1000 (640-1100)	-
+ 0.1 P + 0.03 Fe	1000 (950-1100)	-
+ 0.2 N + 0.03 Fe	44 (41-49)	-
Supernate + phosphate	1900 (1700-2200)[c]	61.
+ 0.05 P	2000 (1700-2400)	-
+ 0.5 N	2600 (2400-2900)	-
+ 0.05 P + 0.5 N	2700 (2300-2900)	-
+ 0.05 P + 0.5 N + 0.03 Fe	2400 (1900-2800)	-
+ 0.1 P + 0.03 Fe	2000 (2100-2800)	-
+ 0.2 N + 0.03 Fe	2700 (2400-3400)	-

[a] Based on 3 determinations except as noted.
[b] Based on 2 determinations.
[c] Based on 5 determinations.

growth potential and, after treatment, algal growth in the samples was stimulated by phosphorus additions, not nitrogen. Addition of phosphorus to near original levels in treated waters resulted in substantial recovery of growth potential, and the reconstituted samples showed algal response to added nitrogen.

The experiment suggested that phosphorus limitation (at least for AAP organisms) can be induced in Snake Lake waters by Al(III) treatment at a concentration of about 10 mg/l.

Mirror Lake water (March, 1972) - An assay technique that would show changes in growth rates was used for Mirror Lake. The data have implications for the Snake Lake results. A volume of autoclaved lake water was treated with 10 mg Al(III)/l, settled, and decanted. Reactive-P and fluorometric growth response were determined on autoclaved, supernatant, and supernatant-plus-nutrients samples as shown in Table 37.

Table 37. ALGAL RESPONSE AND PHOSPHORUS CONTENT OF ALUMINUM (III)-TREATED MIRROR LAKE WATERS

	Fluorometric response units (mean)			Reactive P
	Day 4	Day 7	Day 9	mg/l
Autoclaved lake water	6800	14000	14000	0.52
Supernatant treated water	1500	1200	890	0.08
Supernate + NAAM (-P) components	1300	880	510	0.08
Supernate + 0.44 mg/l P	3300	12000	11000	0.52
Supernate + phosphate and nitrate	3000	15000	15000	0.52
Supernate + 0.44 mg/l P + NAAM (-P)	6300	15000	13000	0.52

The lake water was obtained from the 10-12 m depths of Mirror Lake in a strata which had apparently not been mixed during fall overturn, resulting in high (0.52 mg/l) phosphorus values.

The underlined fluorometer responses in Table 37 should be used for comparison since they represent maximum growth since the maximum growth occurs at a later time when larger amounts of assimilative nutrients are available. The data show a growth response, principally to phosphorus, in the treated waters, with a minor increase when both nitrogen and phosphorus were added.

Bioassay data for the four lakes treated with aluminum are given in Figures 20 to 49 and are summarized in Tables 38 and 39.

The data given for Horseshoe Lake are all post-treatment assays, so no direct evidence of the effect of the treatment is provided by the bioassays. A detailed description of the Horseshoe effort is given by Peterson *et al.*[1] They reported generally positive results of the treatment and identified reduced levels of phosphorus in the hypolimnion during the summers of 1970-72 as one measure of the effectiveness of aluminum treatment. Unfortunately, bioassay data for periods of summer stratification are lacking for the most part, but high algal production in hypolimnetic samples during 1973, along with the occurrence of nitrogen limitation in these samples, may indicate a progressive return to pretreatment conditions. Additional sampling will be required to establish this with certainty.

Horseshoe Lake has a mean hydraulic residence time of only a about 0.7 years, and at times receives substantial quantities of nutrients in runoff from the watershed; both of these factors would tend to shorten the time during which a single application of aluminum would influence the lake. However, even if the lake is reverting to its pretreatment condition, as suggested by the data for the summer of 1973, four summers have elapsed since the lake was treated. This result gives some insight into the permanence which might be expected from aluminum treatment in hardwater lakes similar to Horseshoe.

Repetitive assays for the north and south basins of Snake Lake were initiated in the fall of 1971. Although it was not possible to obtain a full year of pretreatment data--the lake was treated in May, 1972, it is clear that aluminum treatment has reduced the overall fertility of Snake Lake. Comparison of fall and spring data before and after treatment shows approximately a tenfold reduction in biomass produced in both hypolimnetic and epilimnetic samples. In addition, no samples were nitrogen limited since the time of treatment. Prior to that time, none of the samples were phosphorus limited.

Table 38. AVERAGE ALGAL BIOMASS PRODUCED IN SAMPLES FROM LAKES TREATED WITH ALUMINUM[a]

		Epilimnion				Hypolimnion			
Lake	Year	Dec-Feb	Mar-May	June-Aug	Sept-Nov	Dec-Feb	Mar-May	June-Aug	Sept-Nov
Horseshoe, treated 5/70	1971				0.60				1.8
	1972	4.4	220.		0.76	6.0	8.6		38.
	1973	20.	11.	1.4		22.	18.	57.	
Snake (north basin), treated 5/72	1971				47.				260.
	1972	83.	8.9	0.36	1.5	86.	50.	0.96	2.0
	1973	2.1	1.5	1.1		10.	2.0	1.1	
Snake (south basin), treated 5/72	1971								
	1972	90.		0.37	0.64	74.		0.41	0.55
	1973	1.2	0.76	1.8		2.0	1.1	1.1	
Long, treated 5/72	1971				0.45				
	1972	0.64	0.28	0.13	0.17	0.51	0.24	0.095	0.16
	1973	0.38	0.11	0.36		0.22	0.16	0.41	
Pickerel, treated 4/73	1971			0.83	0.19			1.2	0.096
	1972	1.6	0.11	2.1	0.31	2.1	1.2	2.1	0.77
	1973	0.42	0.30	0.26		0.39	0.82	0.30	

[a] All values given in mg/l dry weight.

Table 39. NUTRIENT LIMITATION IN SAMPLES FROM LAKES TREATED WITH ALUMINUM

			Number of samples which responded to additions of:			
Lake	Year	Location	N	P	N&P	Total samples
Horseshoe (treated 5/70)	1971	epi	-	1	1	3
		hypo	-	2	-	3
	1972	epi	-	3	1	6
		hypo	-	3	-	5
	1973	epi	1	4	1	7
		hypo	2	3	2	7
Snake, North Basin (treated 5/72)	1971	epi	2	-	1	3
		hypo	1	-	-	1
	1972	epi	2	5	6	14
		hypo	2	9	2	13
	1973	epi	-	5	5	10
		hypo	-	8	2	10
Snake, South Basin (treated 5/72)	1971	epi	1	-	-	1
		hypo	-	-	-	0
	1972	epi	1	7	-	9
		hypo	1	5	2	9
	1973	epi	-	6	3	10
		hypo	-	10	-	11

Table 39. NUTRIENT LIMITATION IN SAMPLES FROM LAKES TREATED WITH ALUMINUM

Lake	Year	Location	Number of samples which responded to additions of: N	P	N&P	Total samples
Long (treated 5/72)	1971	epi	-	-	1	1
		hypo	-	-	-	0
	1972	epi	-	4	8	12
		hypo	-	5	8	13
	1973	epi	-	3	3	6
		hypo	1	3	2	6
Pickerel (treated 4/73)	1971	epi	-	3	-	4
		hypo	-	2	-	3
	1972	epi	-	10	1	12
		hypo	-	11	1	14
	1973	epi	-	12	1	13
		hypo	-	12	1	13

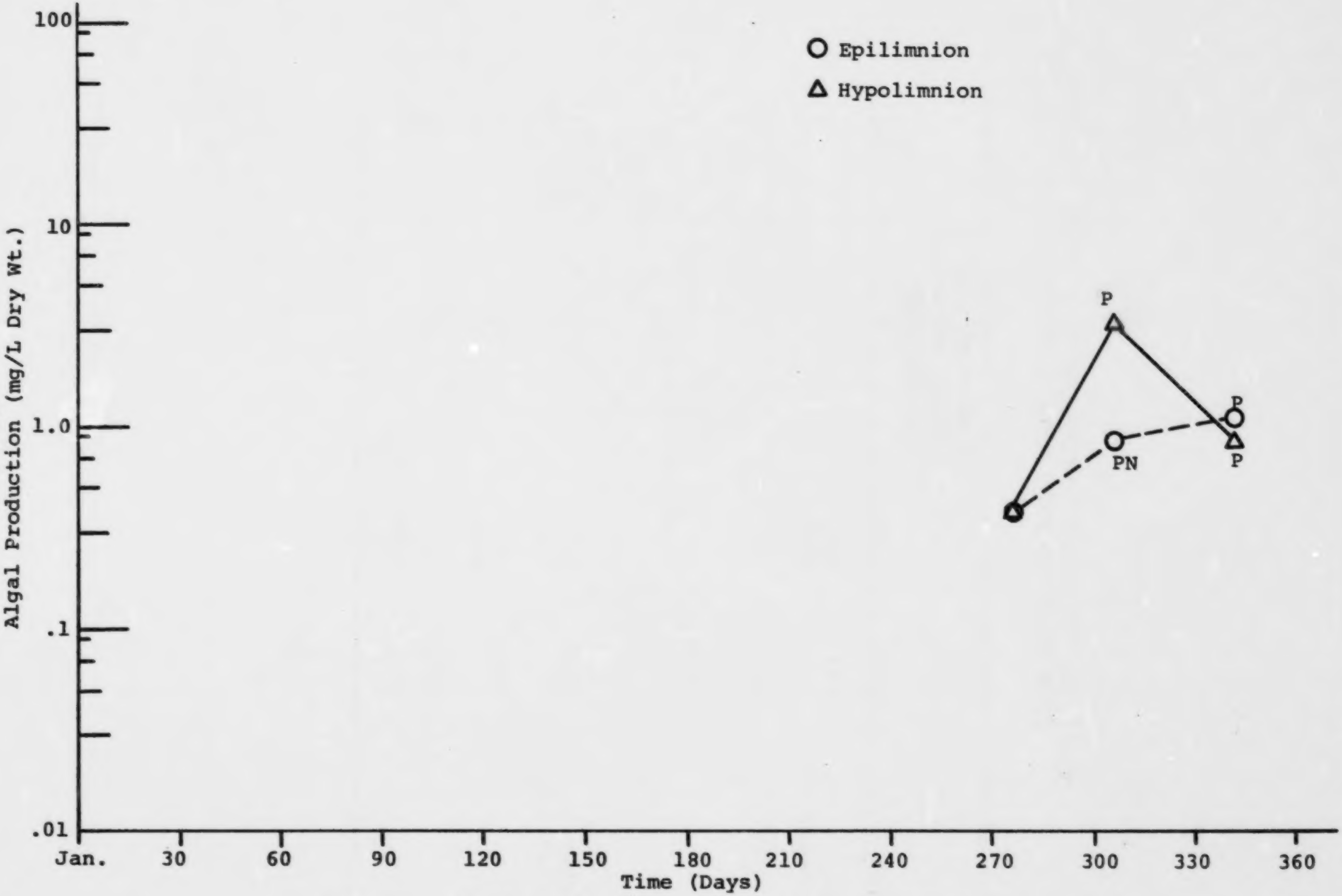

Fig. 20. Algal Production - Horseshoe Lake, 1971

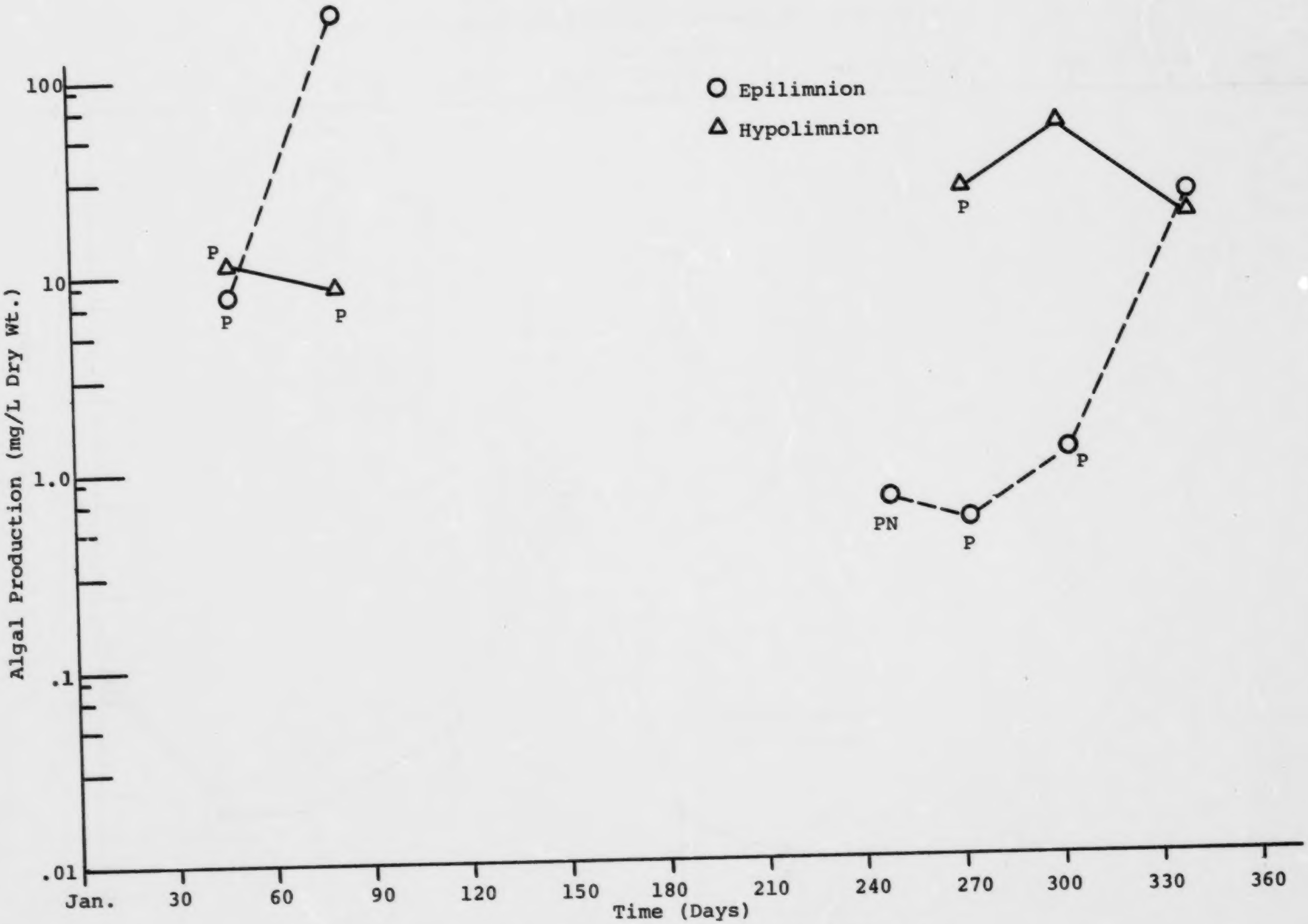

Fig. 21. Algal Production - Horseshoe Lake, 1972

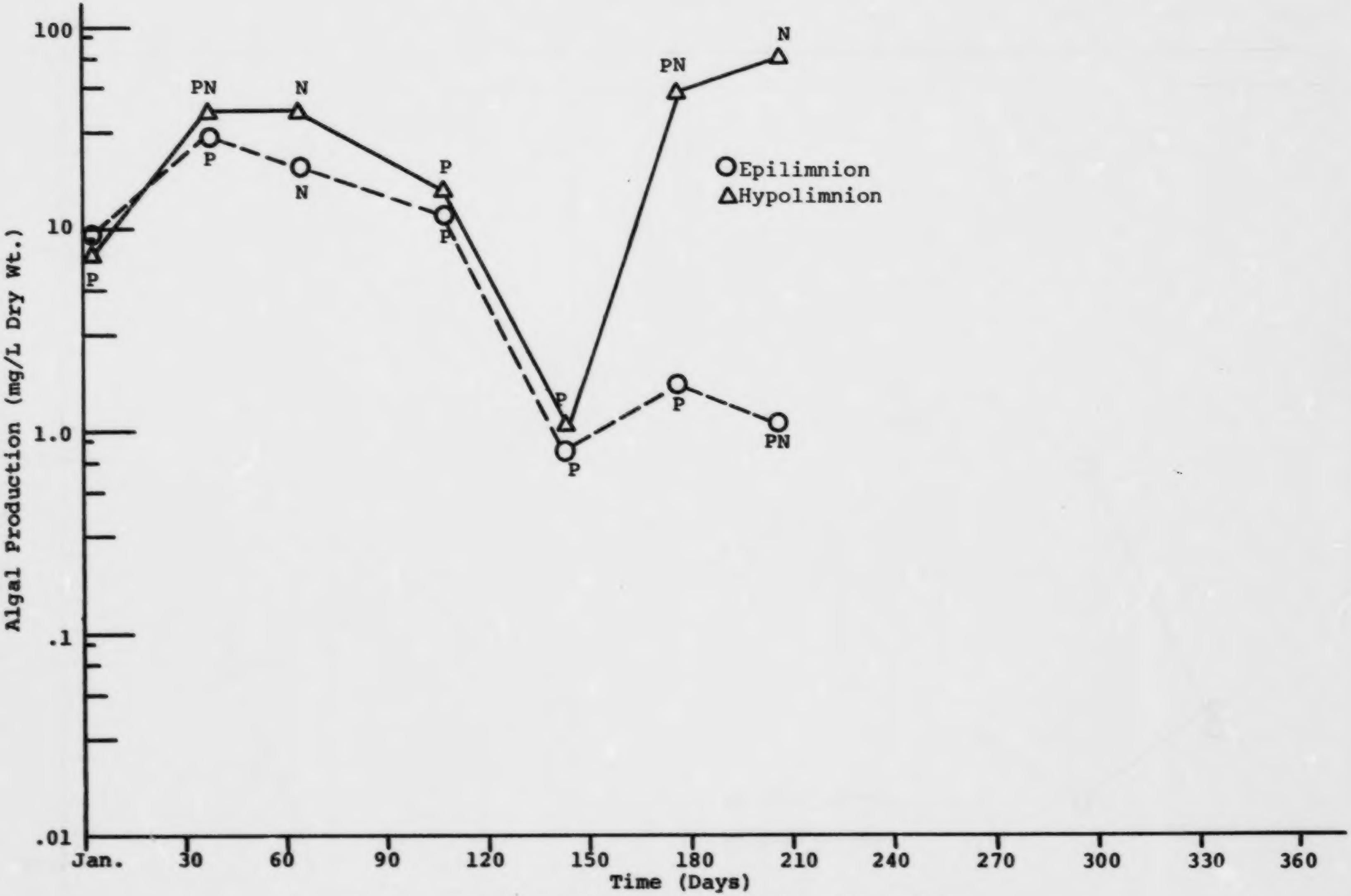

Fig. 22. Algal Production - Horseshoe Lake, 1973

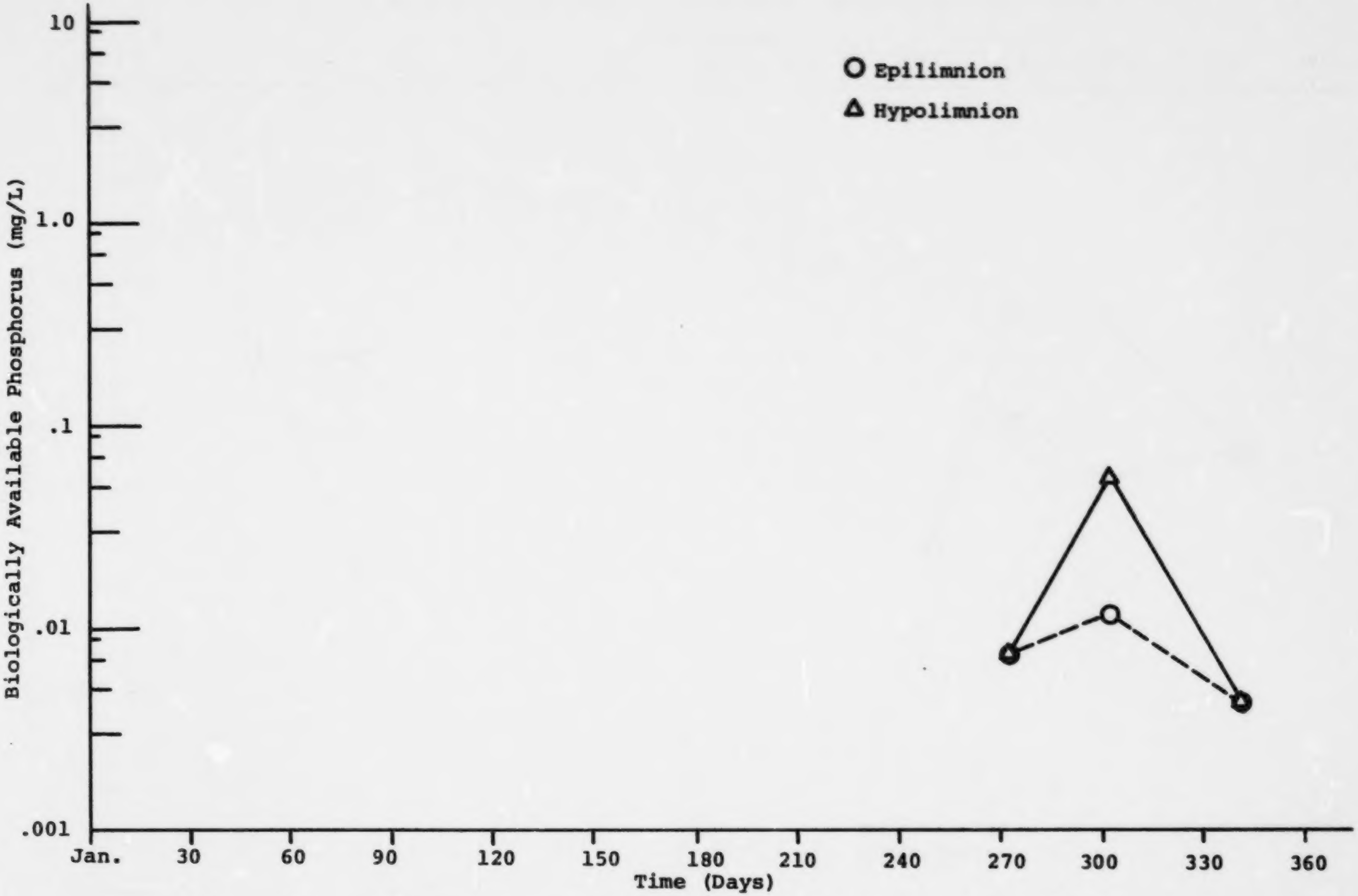

Fig. 23. Biologically Available Phosphorus - Horseshoe Lake, 1971

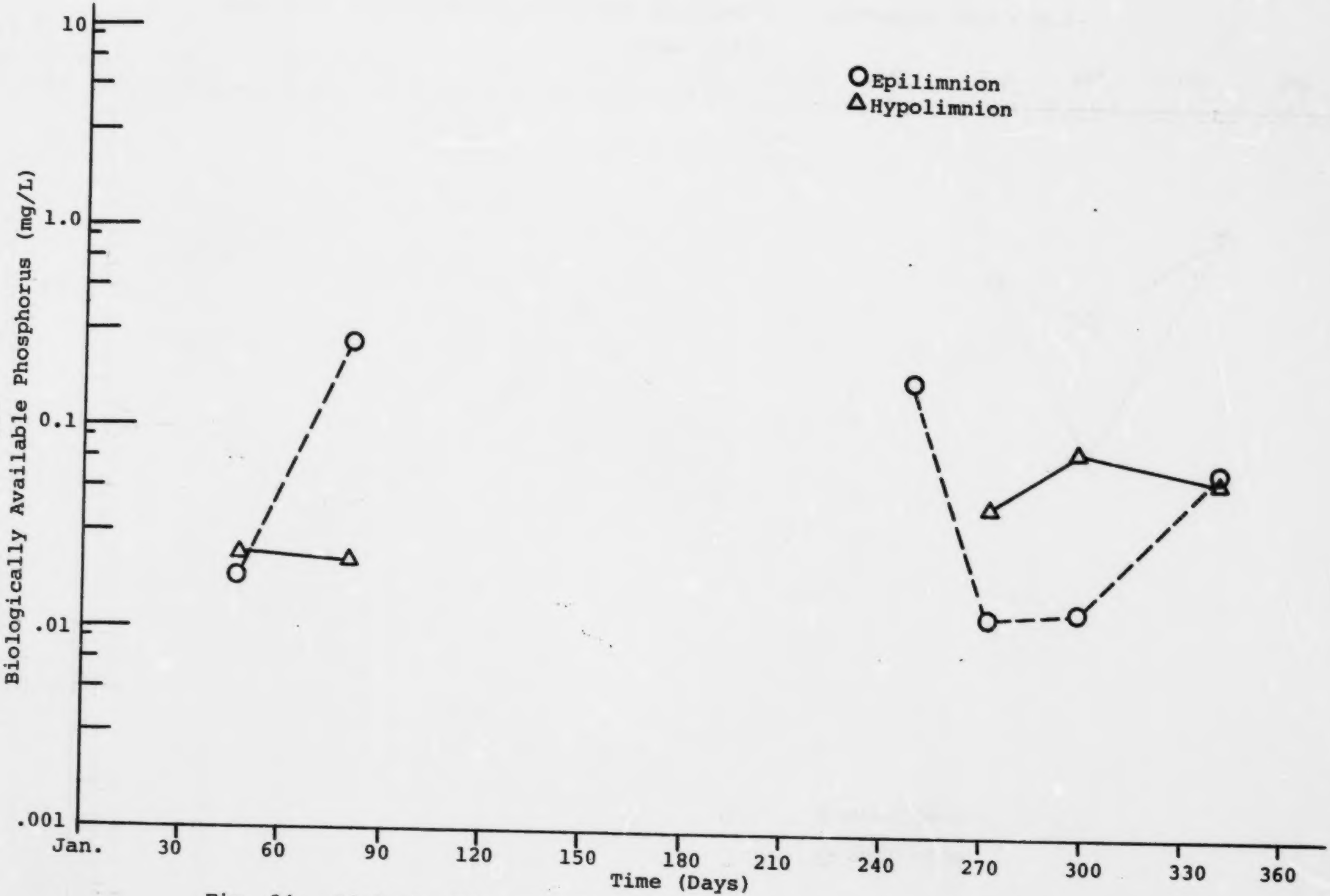

Fig. 24. Biologically Available Phosphorus - Horseshoe Lake, 1972

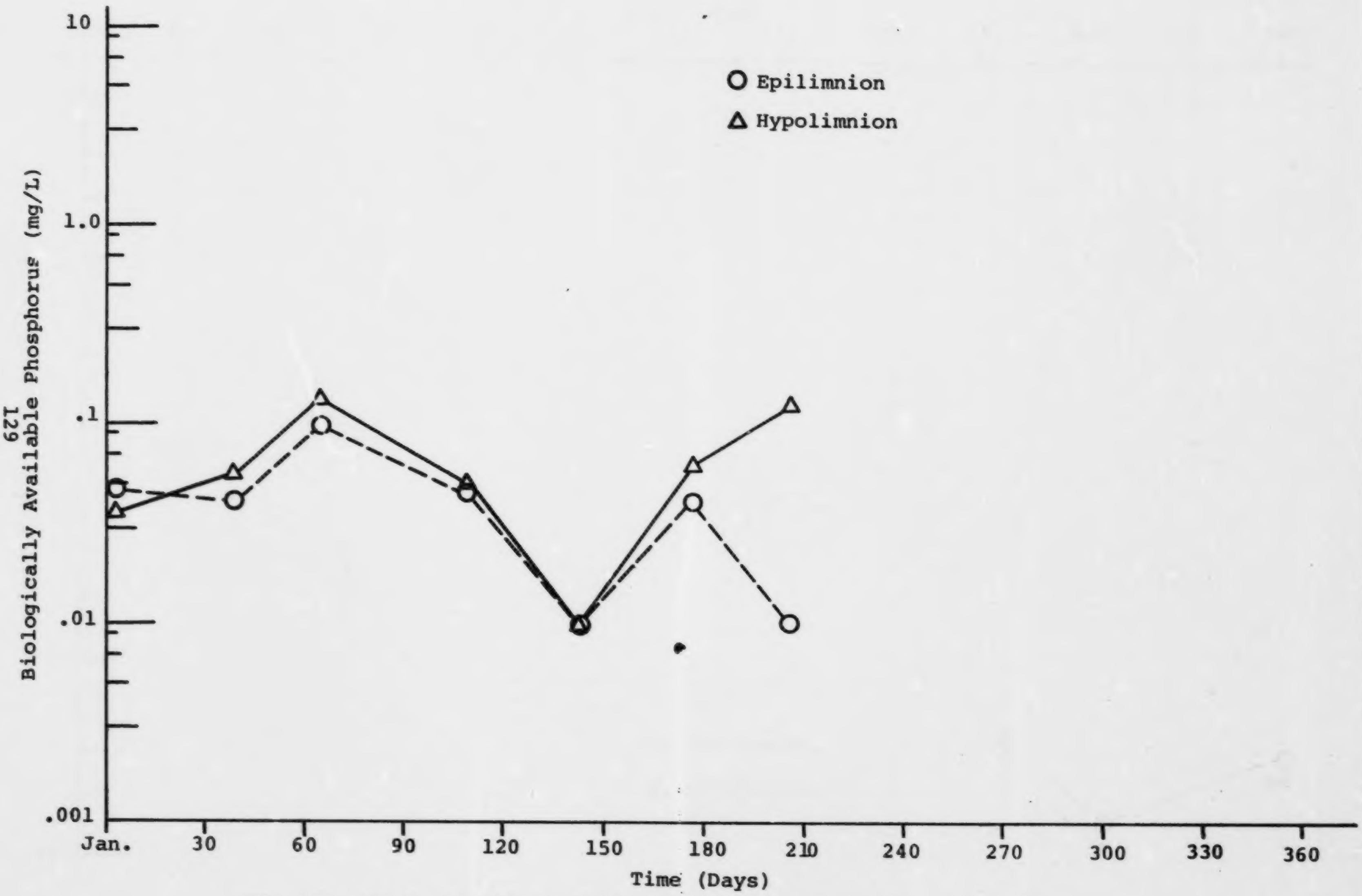

Fig. 25. Biologically Available Phosphorus, Horseshoe Lake, 1973

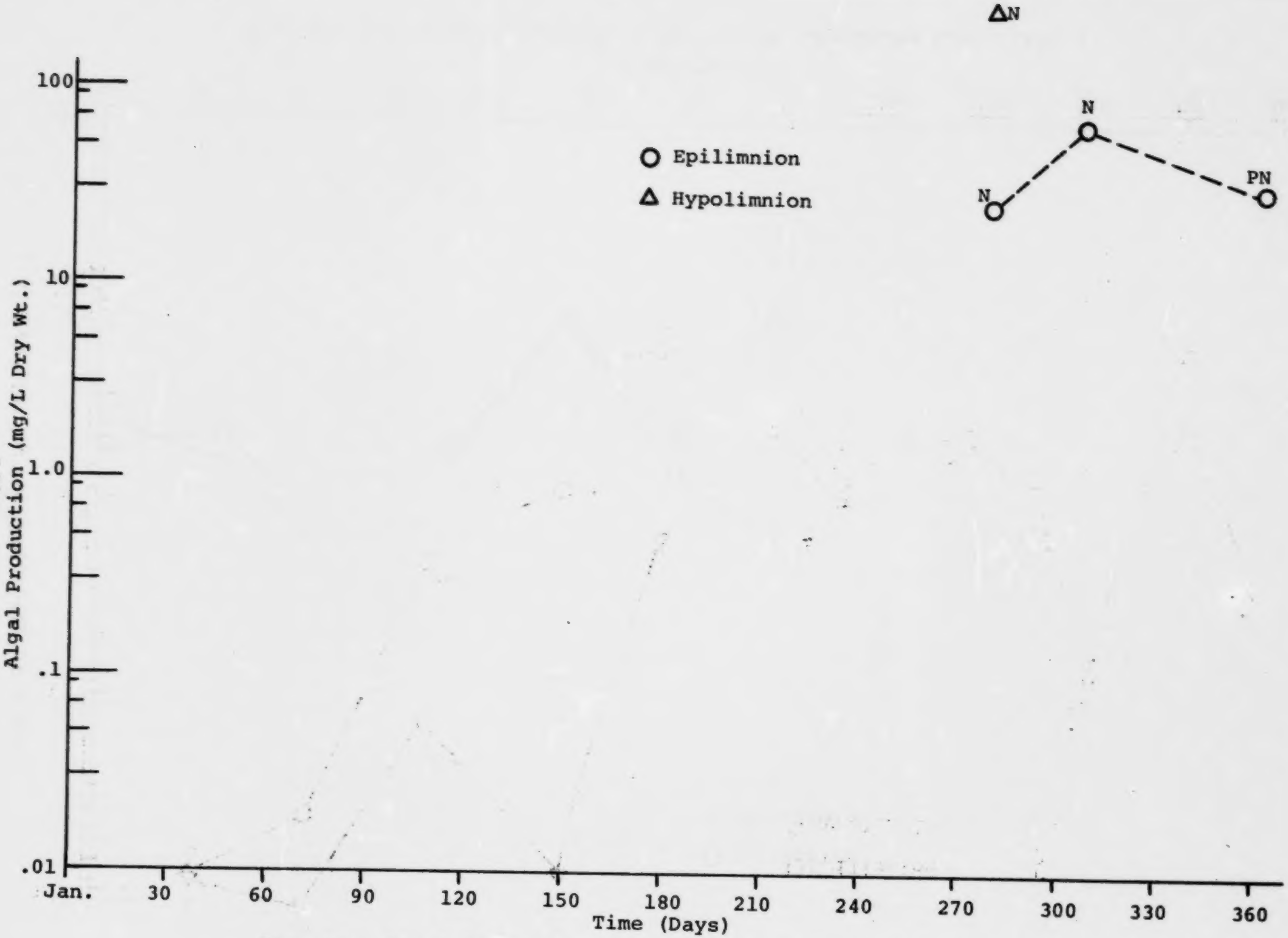

Fig. 26. Algal Production - Snake Lake, North Basin, 1971

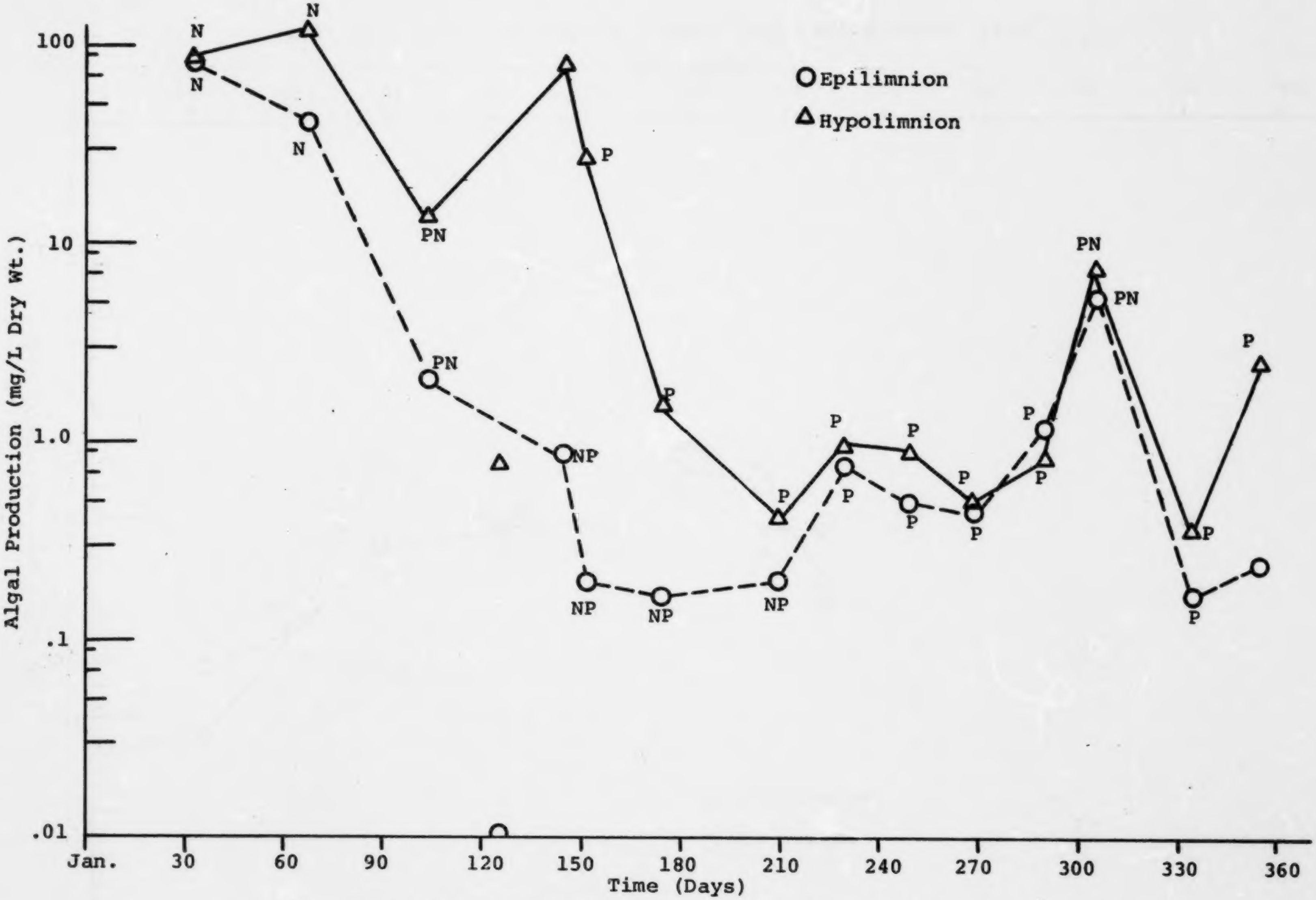

Fig. 27. Algal Production - Snake Lake, North Basin, 1972

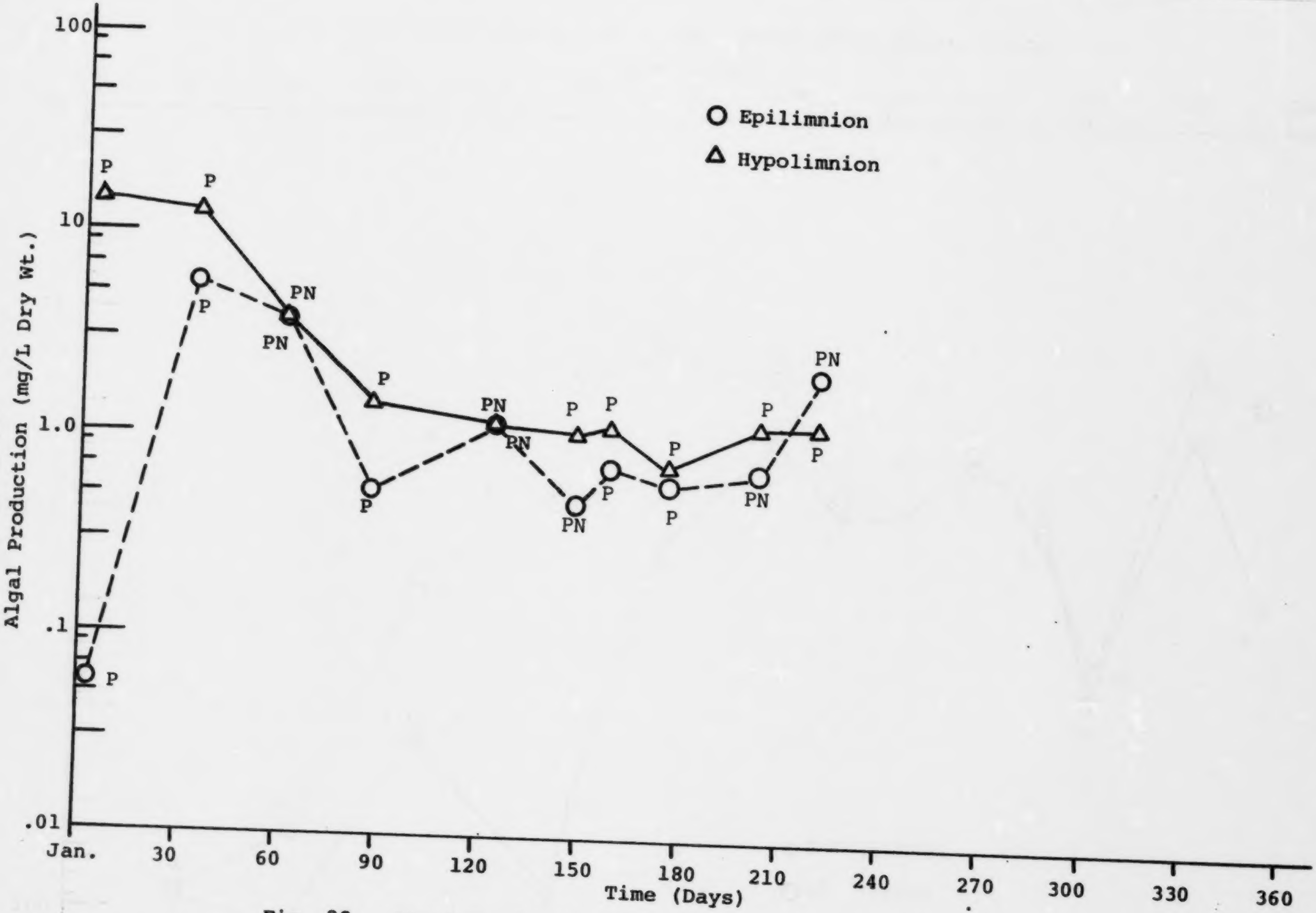

Fig. 28. Algal Production - Snake Lake, North Basin, 1973

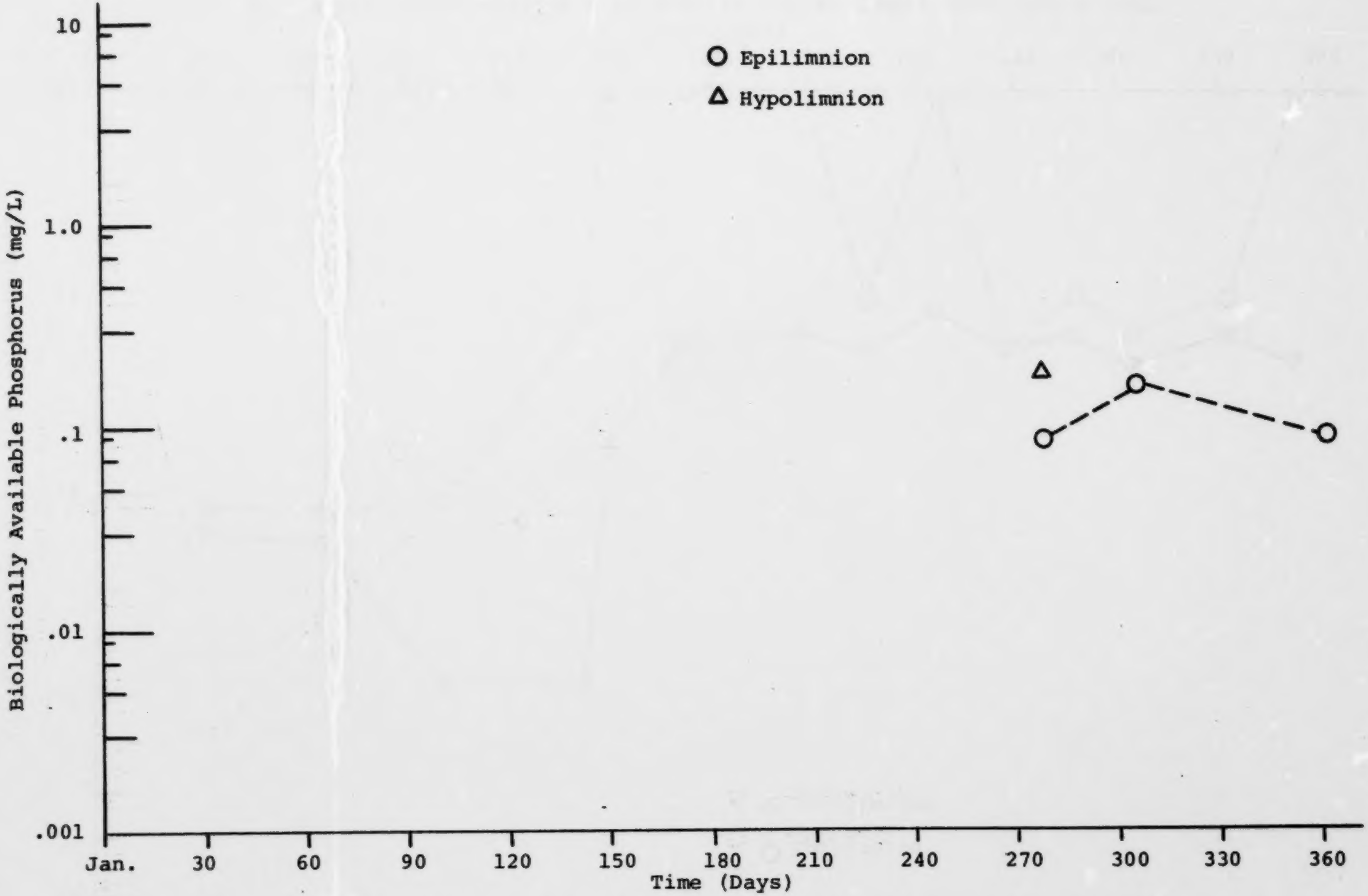

Fig. 29. Biologically Available Phosphorus - Snake Lake, North Basin, 1971

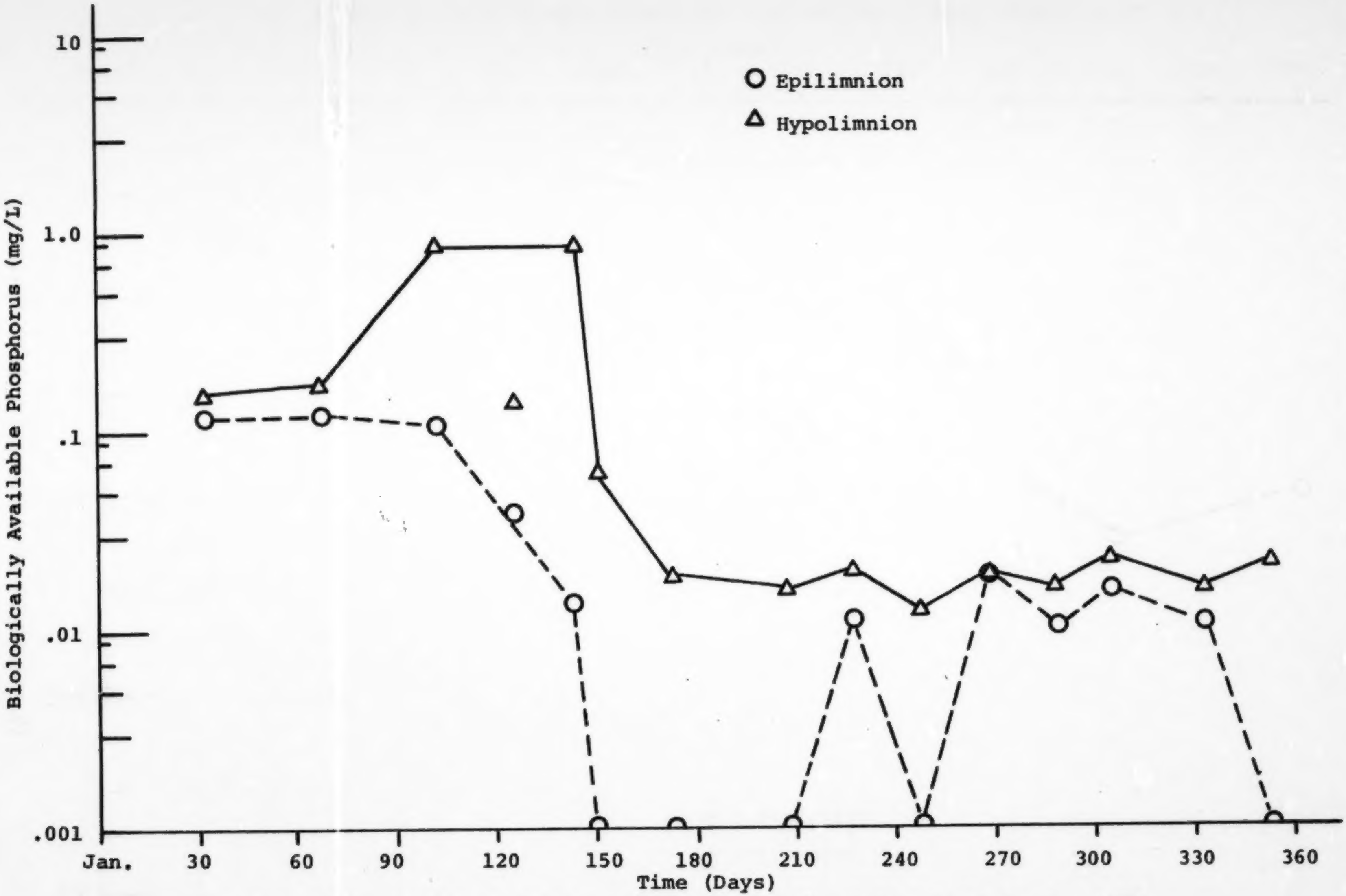

Fig. 30. Biologically Available Phosphorus - Snake Lake, North Basin, 1972

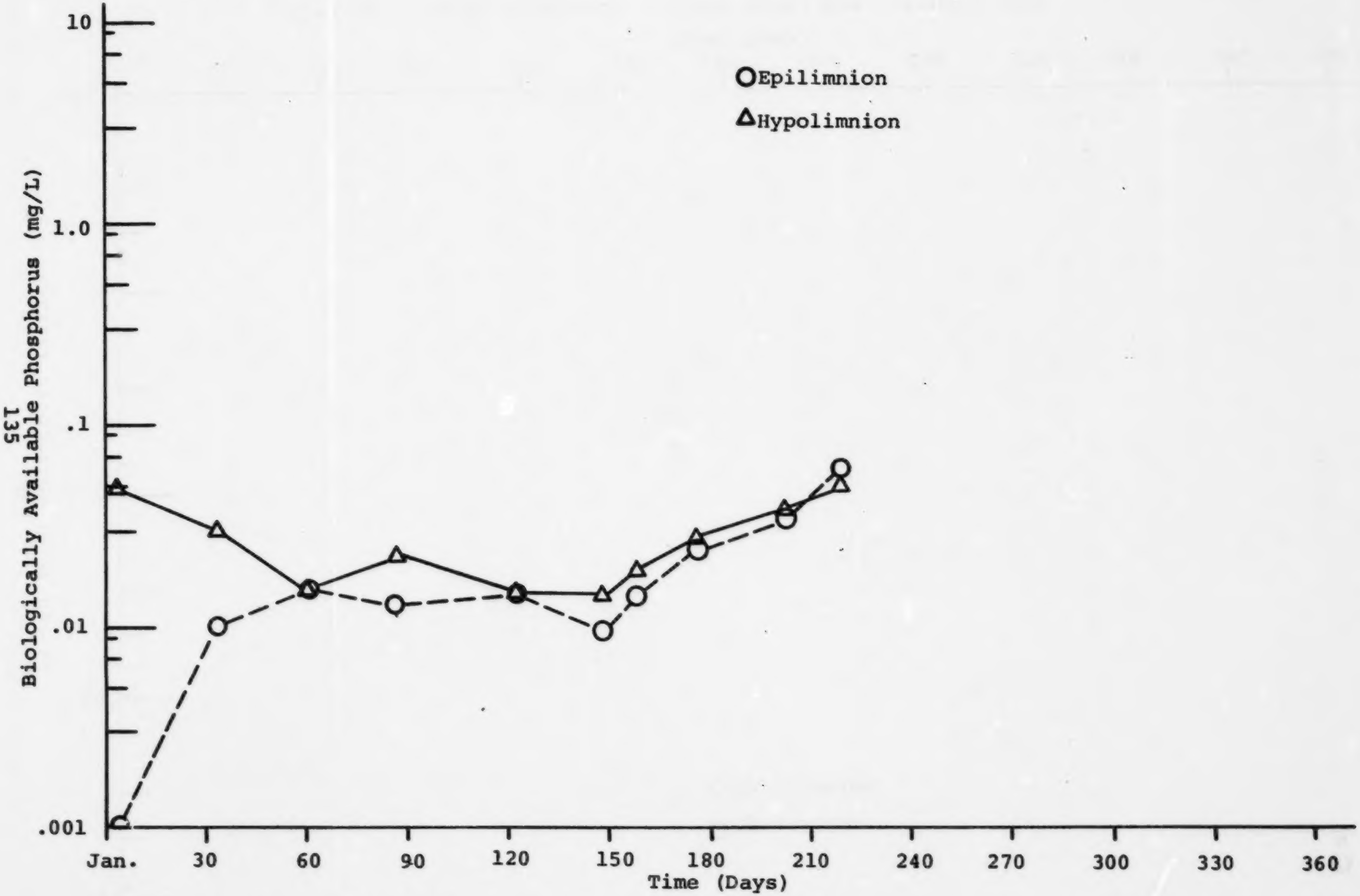

Fig. 31. Biologically Available Phosphorus - Snake Lake, North Basin, 1973

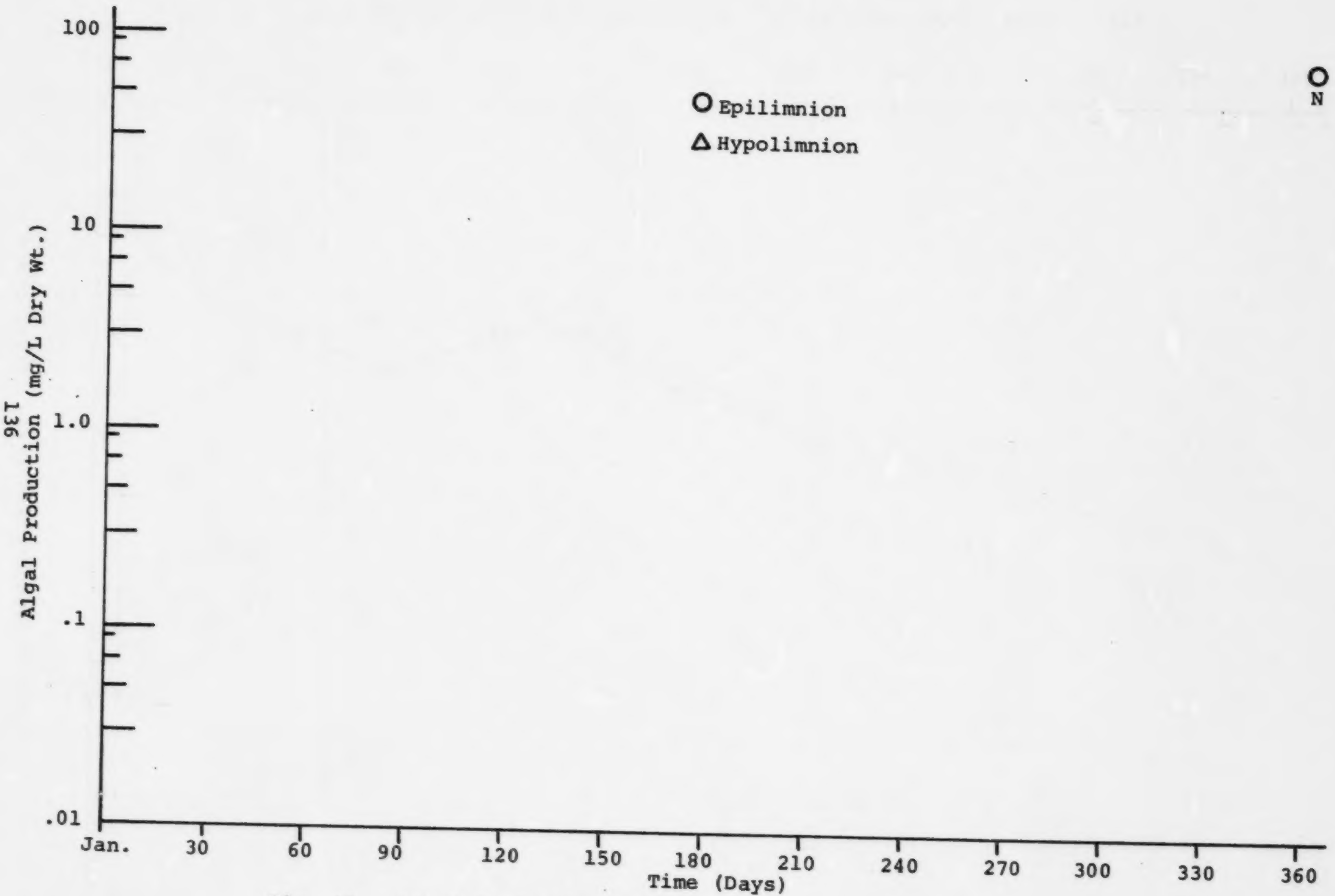

Fig. 32. Algal Production - Snake Lake, South Basin, 1971

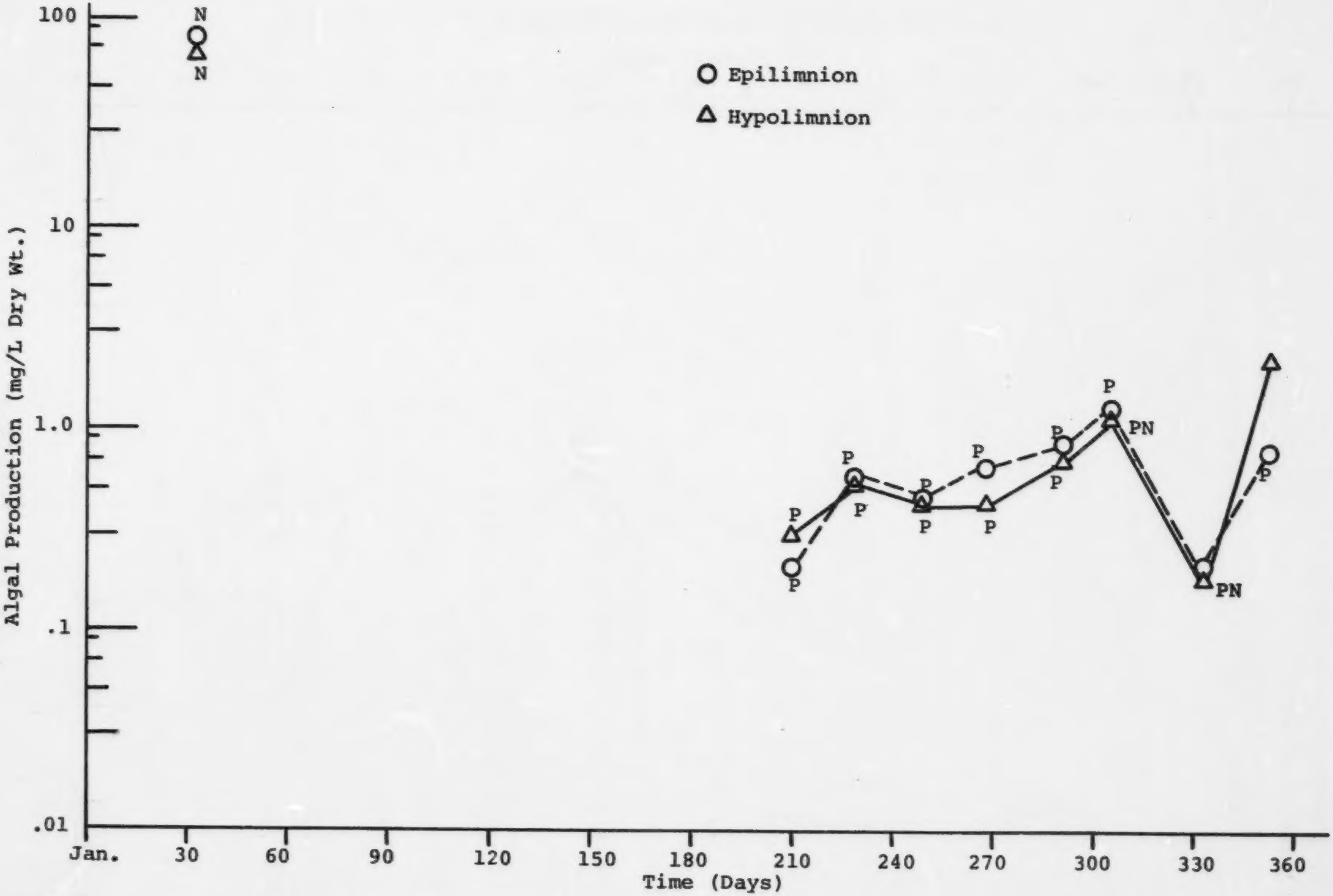

Fig. 33. Algal Production - Snake Lake, South Basin, 1972

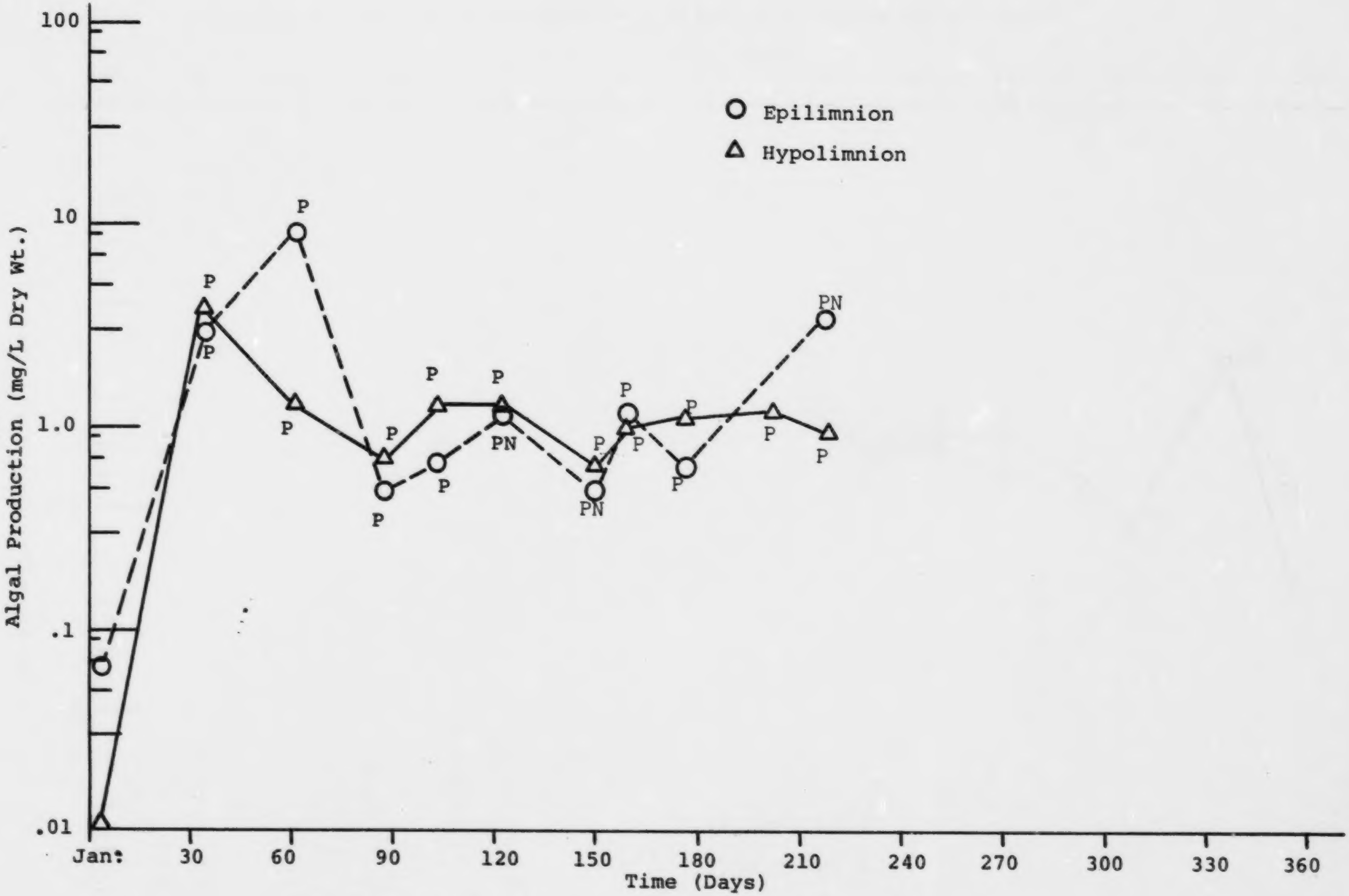

Fig. 34. Algal Production - Snake Lake, South Basin, 1973

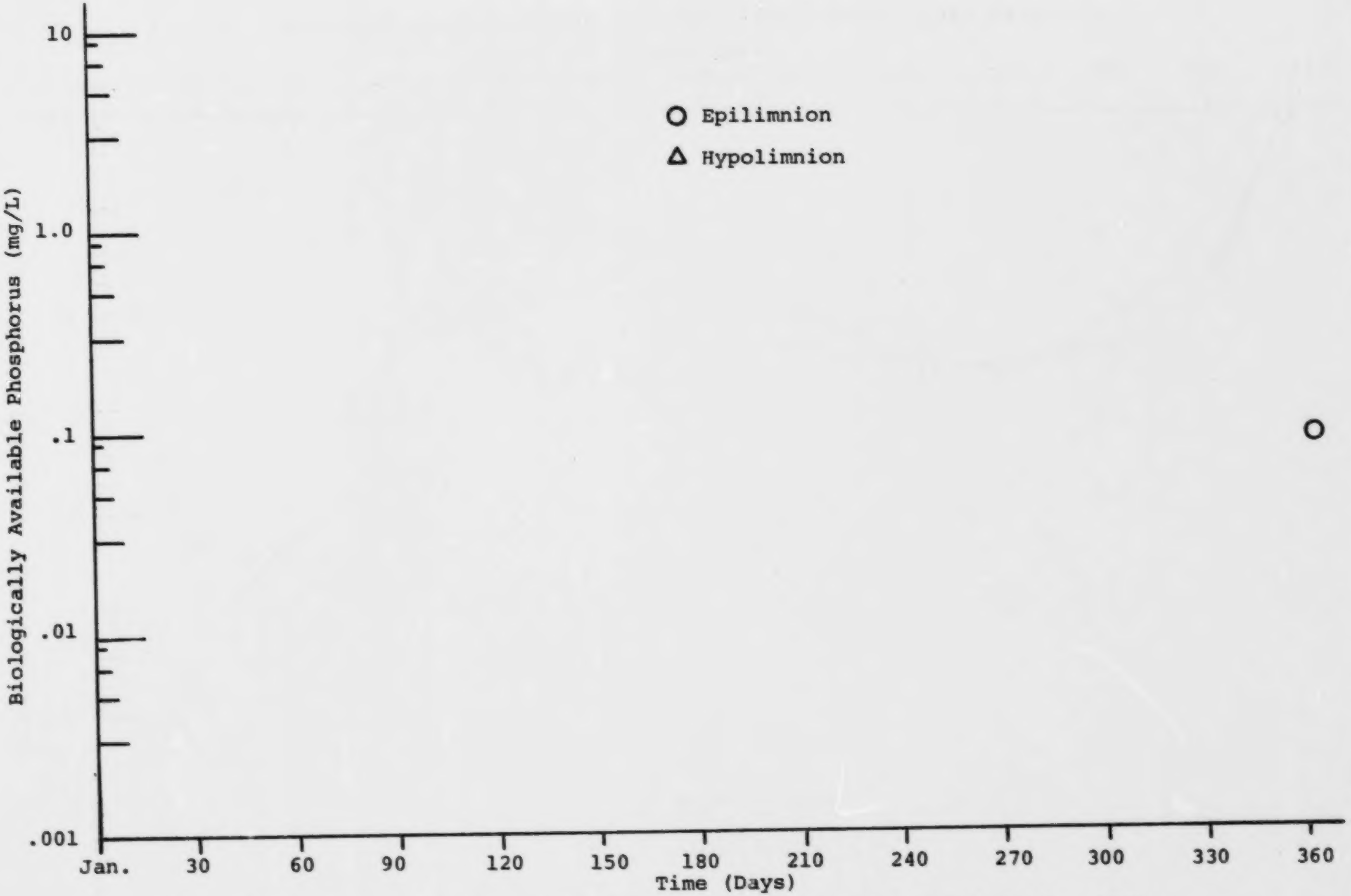

Fig. 35. Biologically Available Phosphorus - Snake Lake, South Basin, 1971

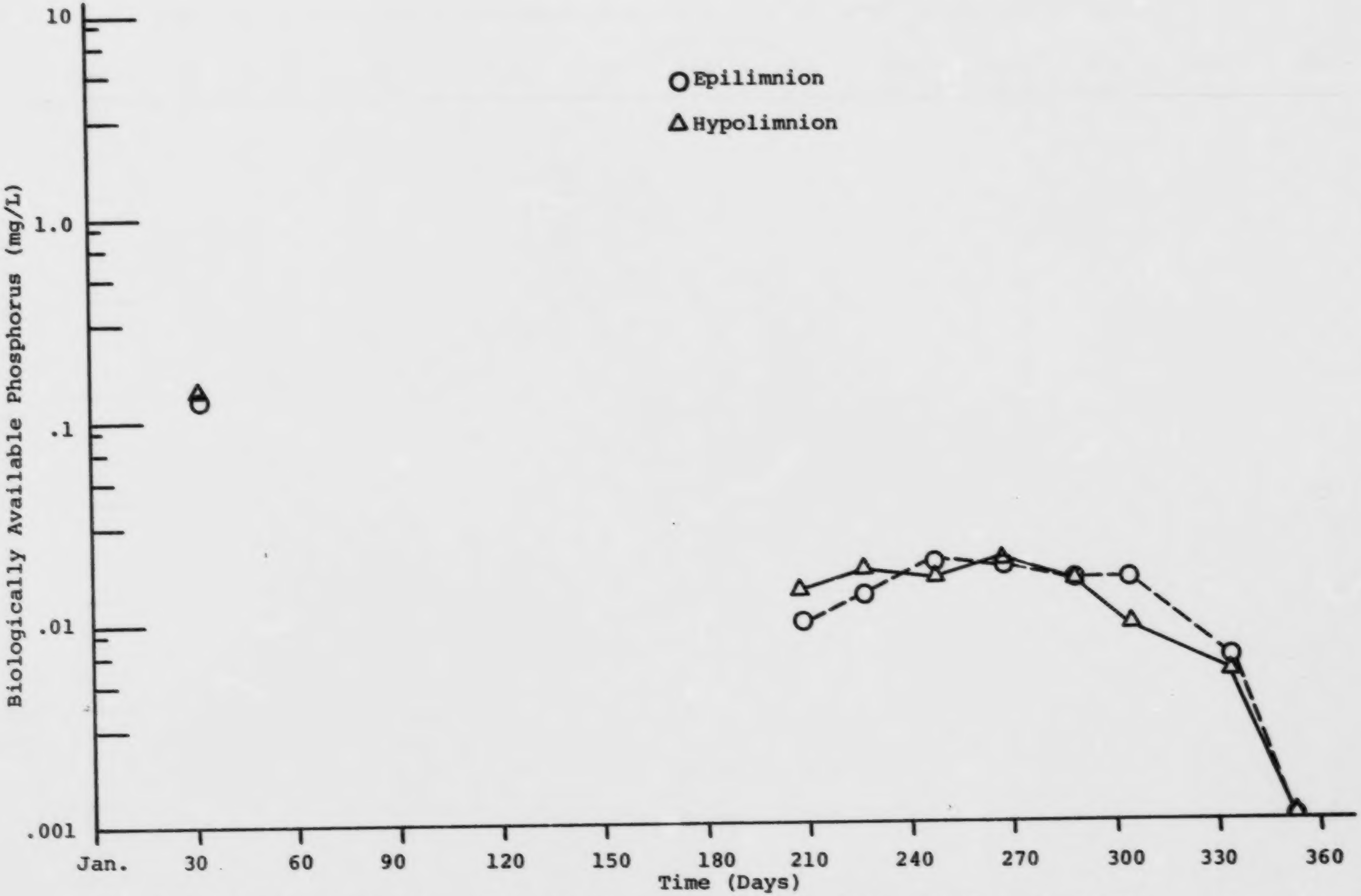

Fig. 36. Biologically Available Phosphorus - Snake Lake, South Basin, 1972

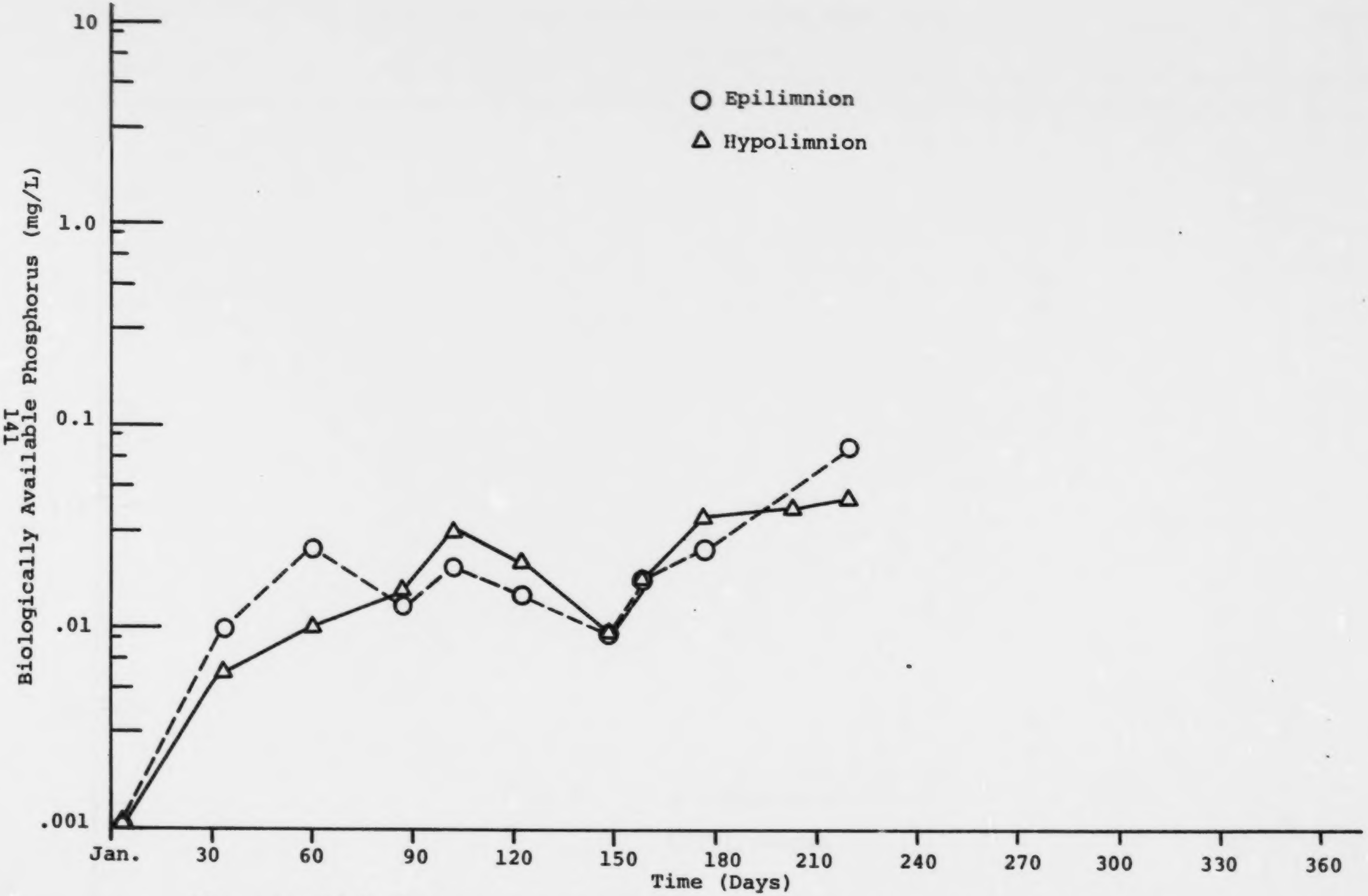

Fig. 37. Biologically Available Phosphorus - Snake Lake, South Basin, 1973

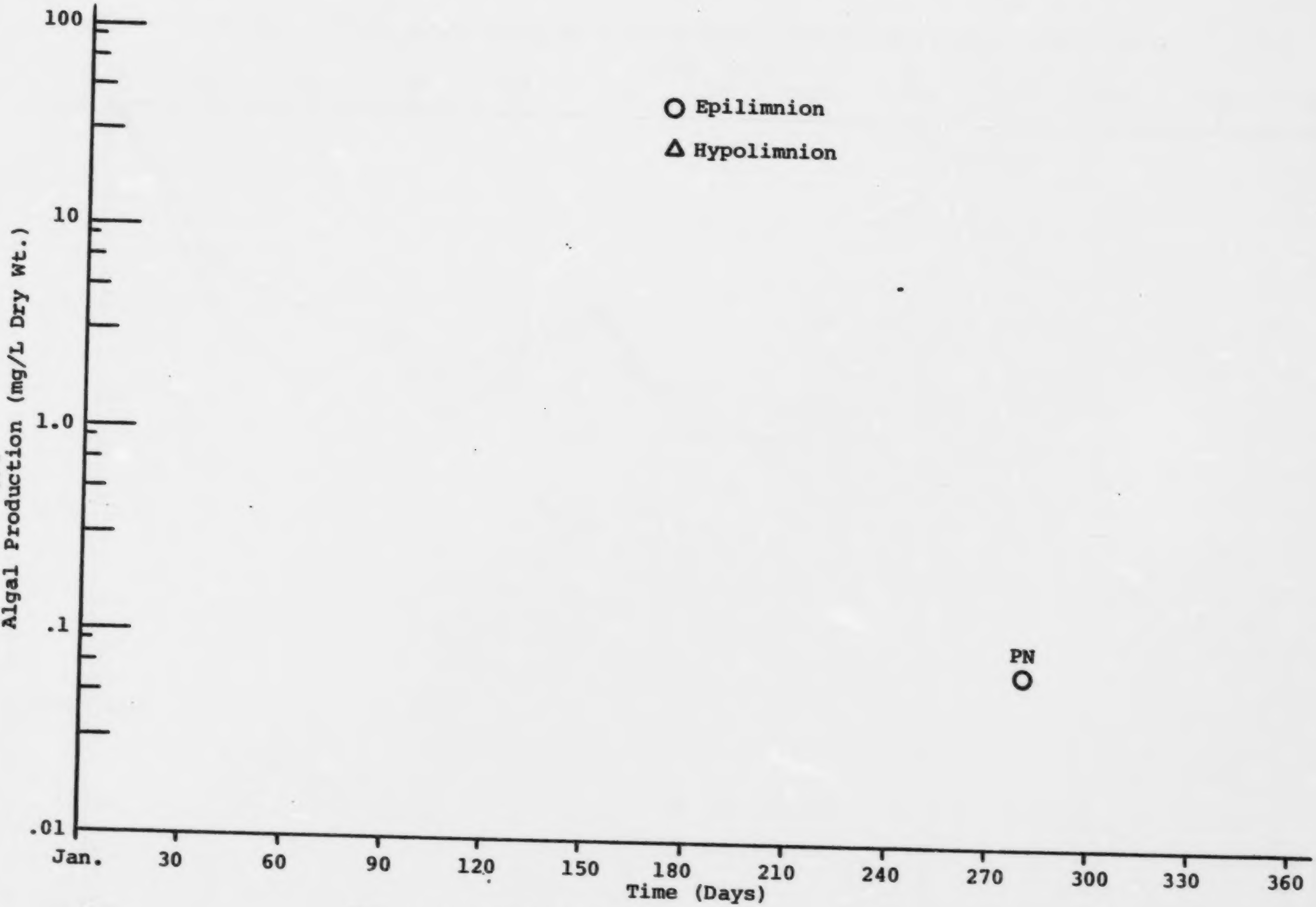

Fig. 38. Algal Production - Long Lake, 1971

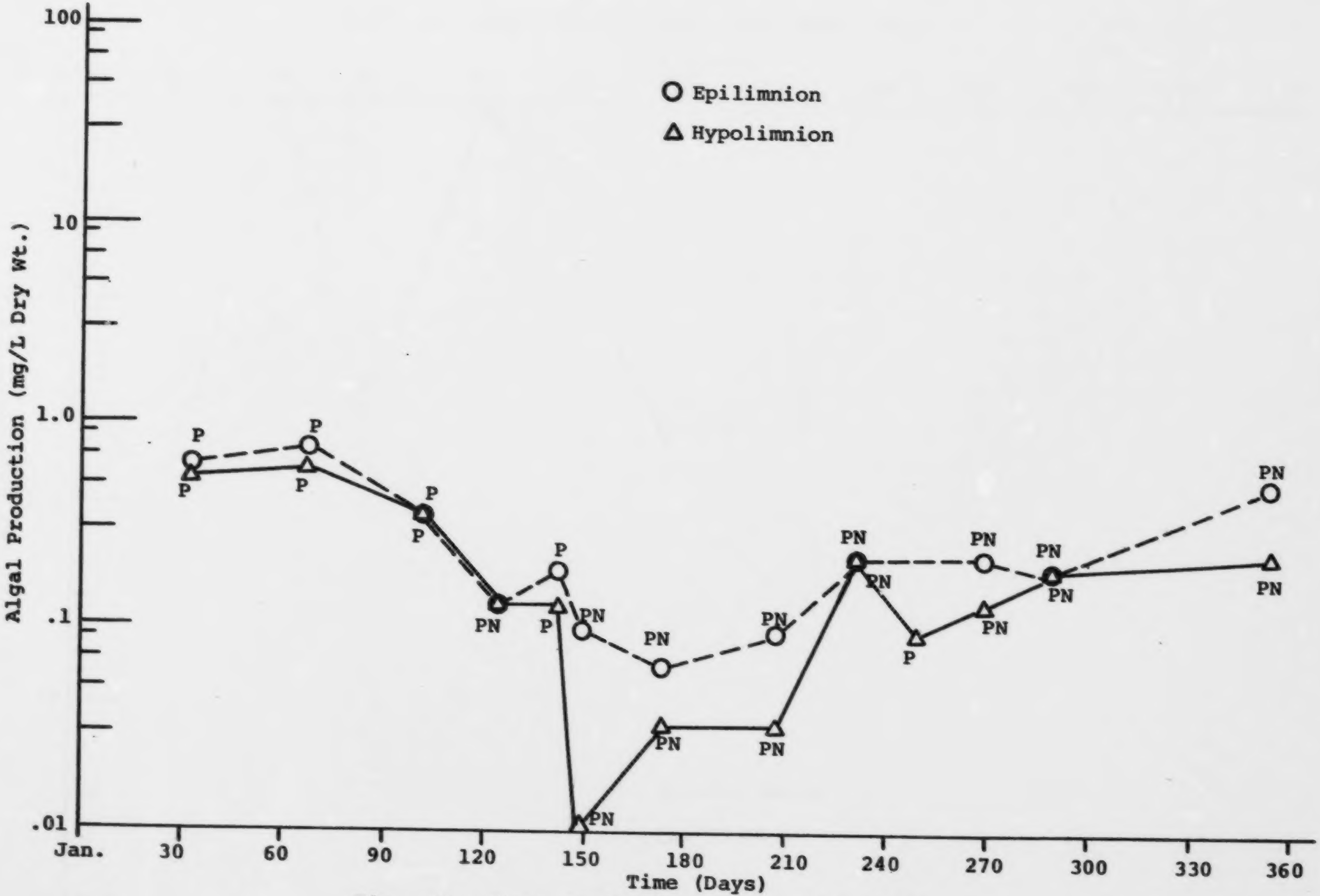

Fig. 39. Algal Production - Long Lake, 1972

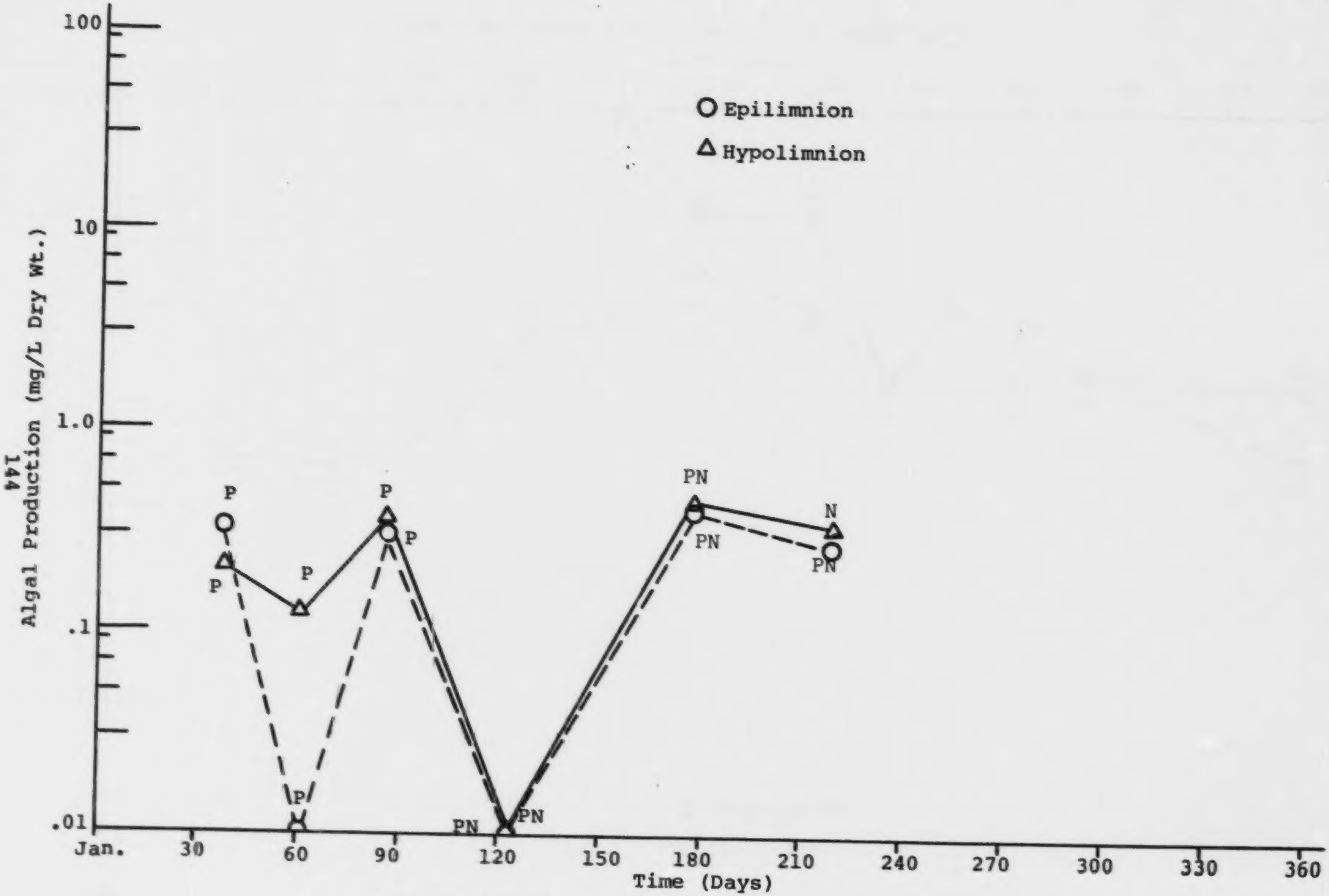

Fig. 40. Algal Production - Long Lake, 1973

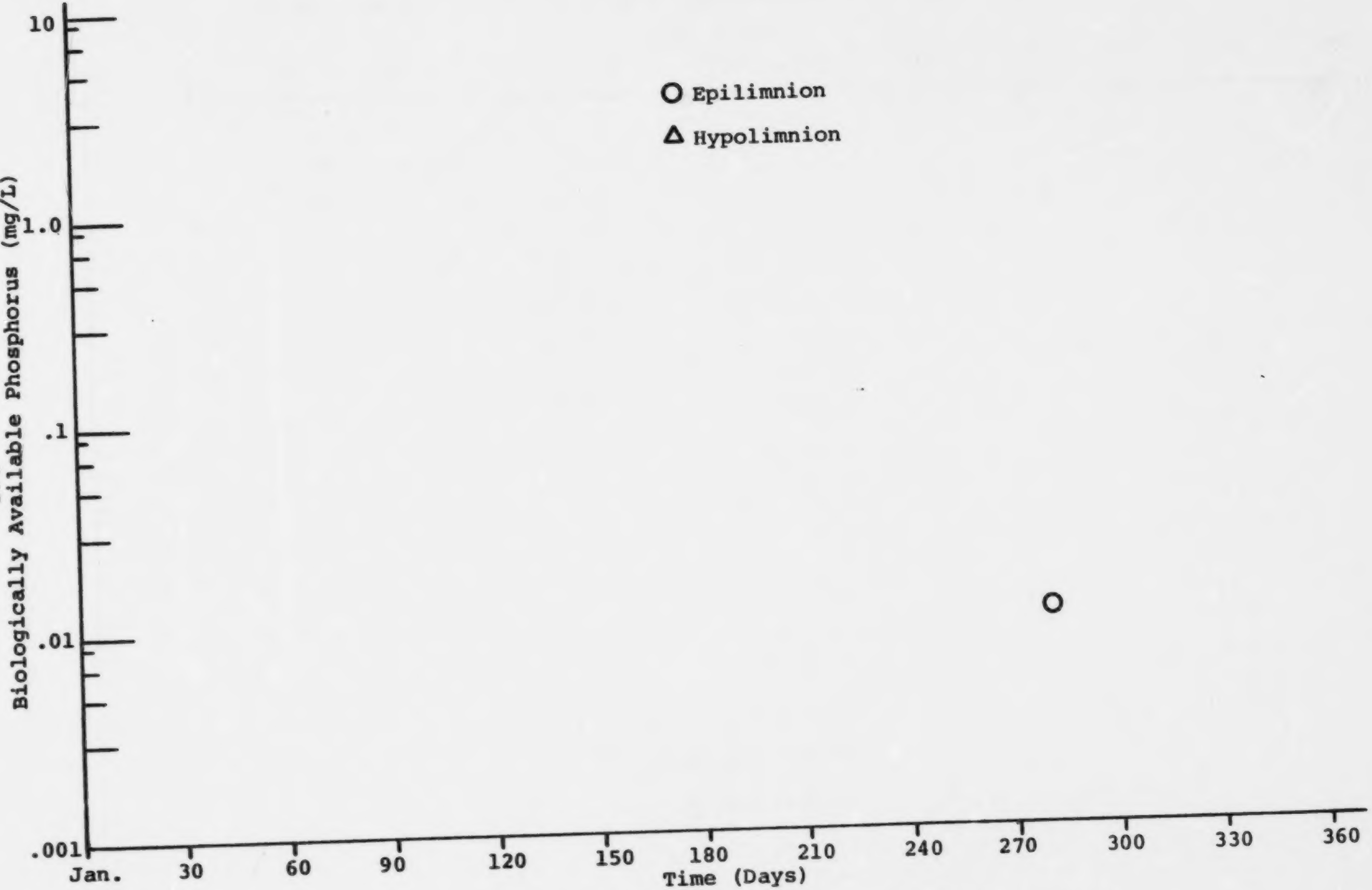

Fig. 41. Biologically Available Phosphorus - Long Lake, 1971

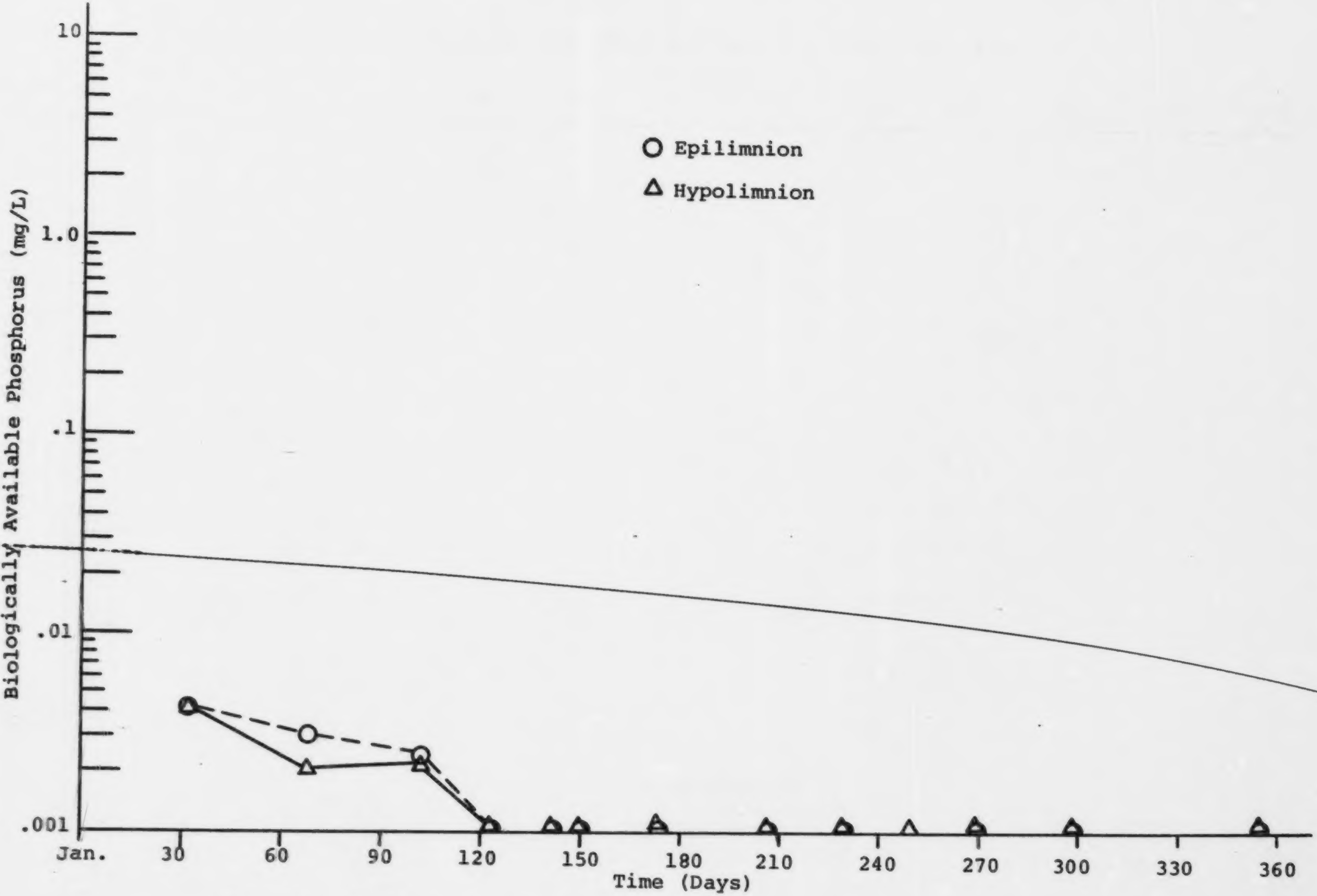

Fig. 42. Biologically Available Phosphorus - Long Lake, 1972

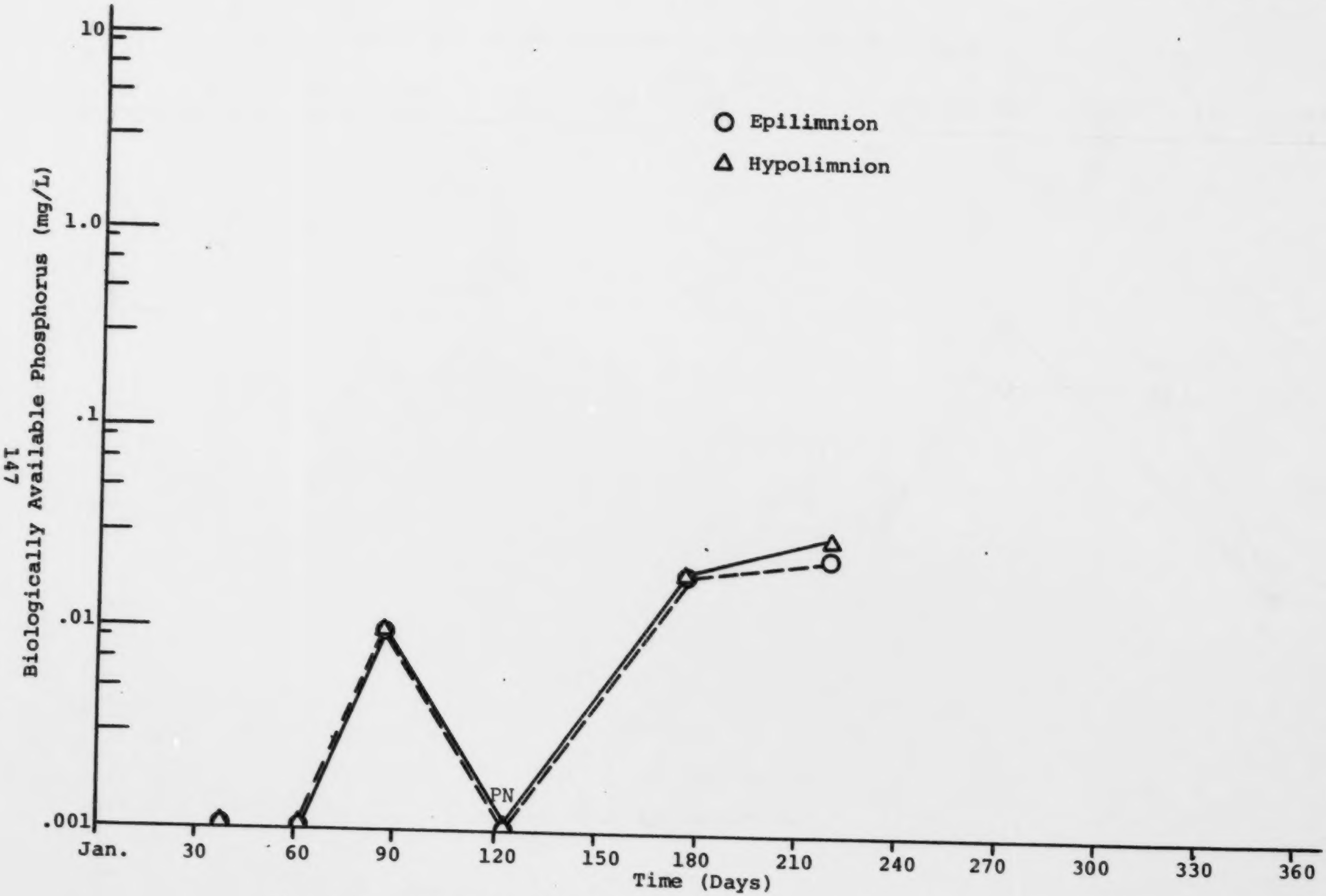

Fig. 43. Biologically Available Phosphorus - Long Lake, 1973

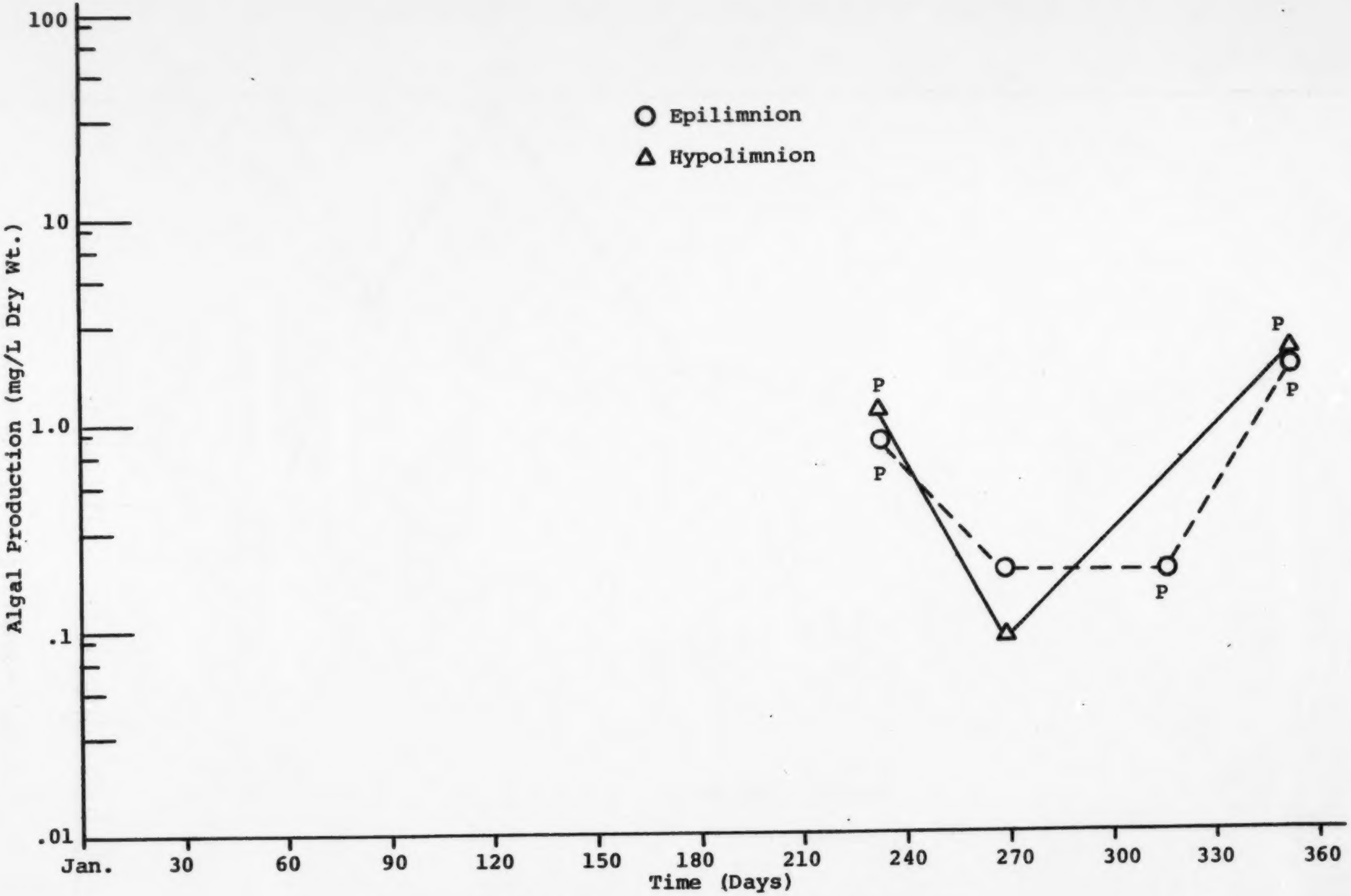

Fig. 44. Algal Production - Pickerel Lake, 1971

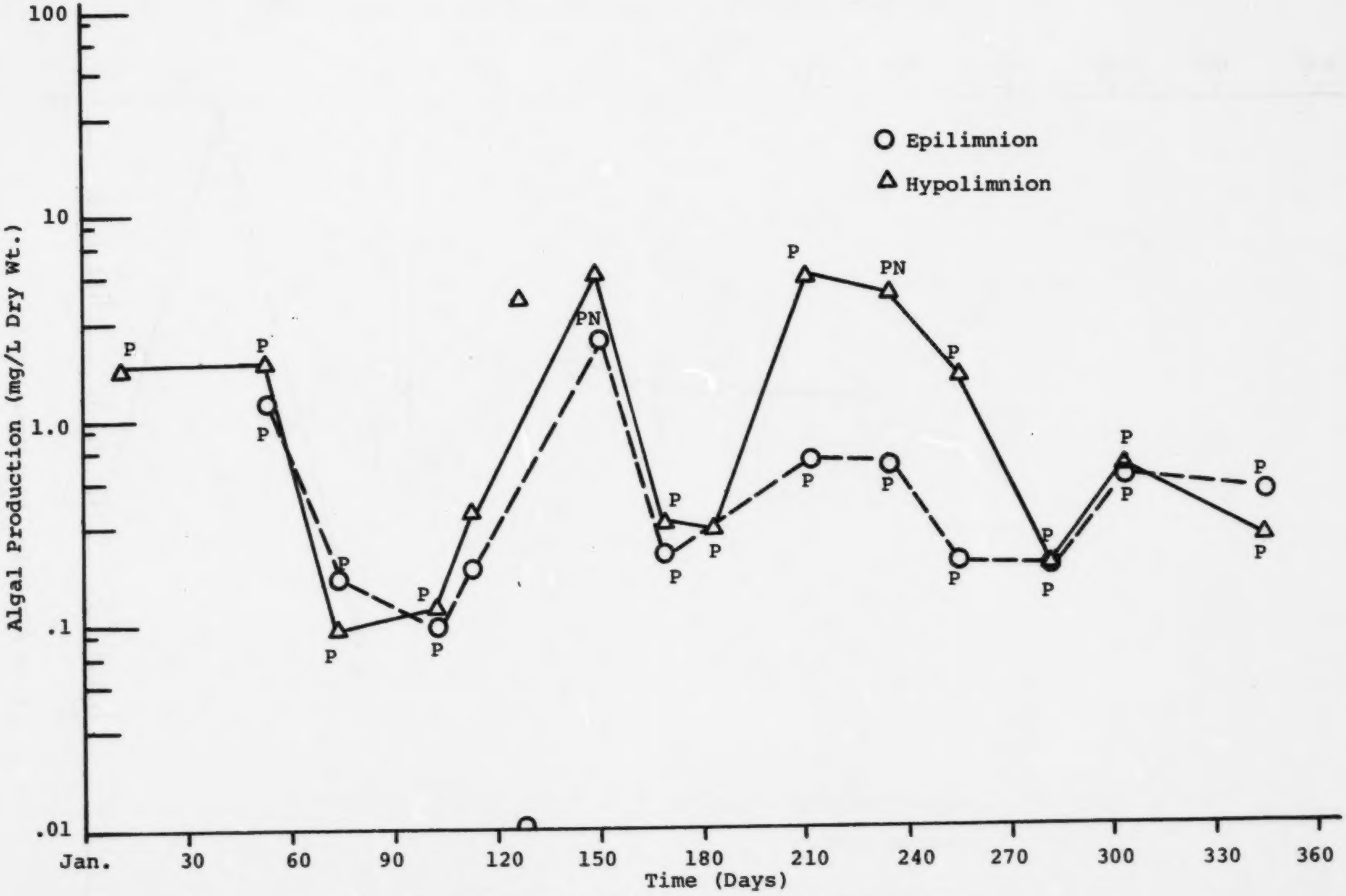

Fig. 45. Algal Production - Pickerel Lake, 1972

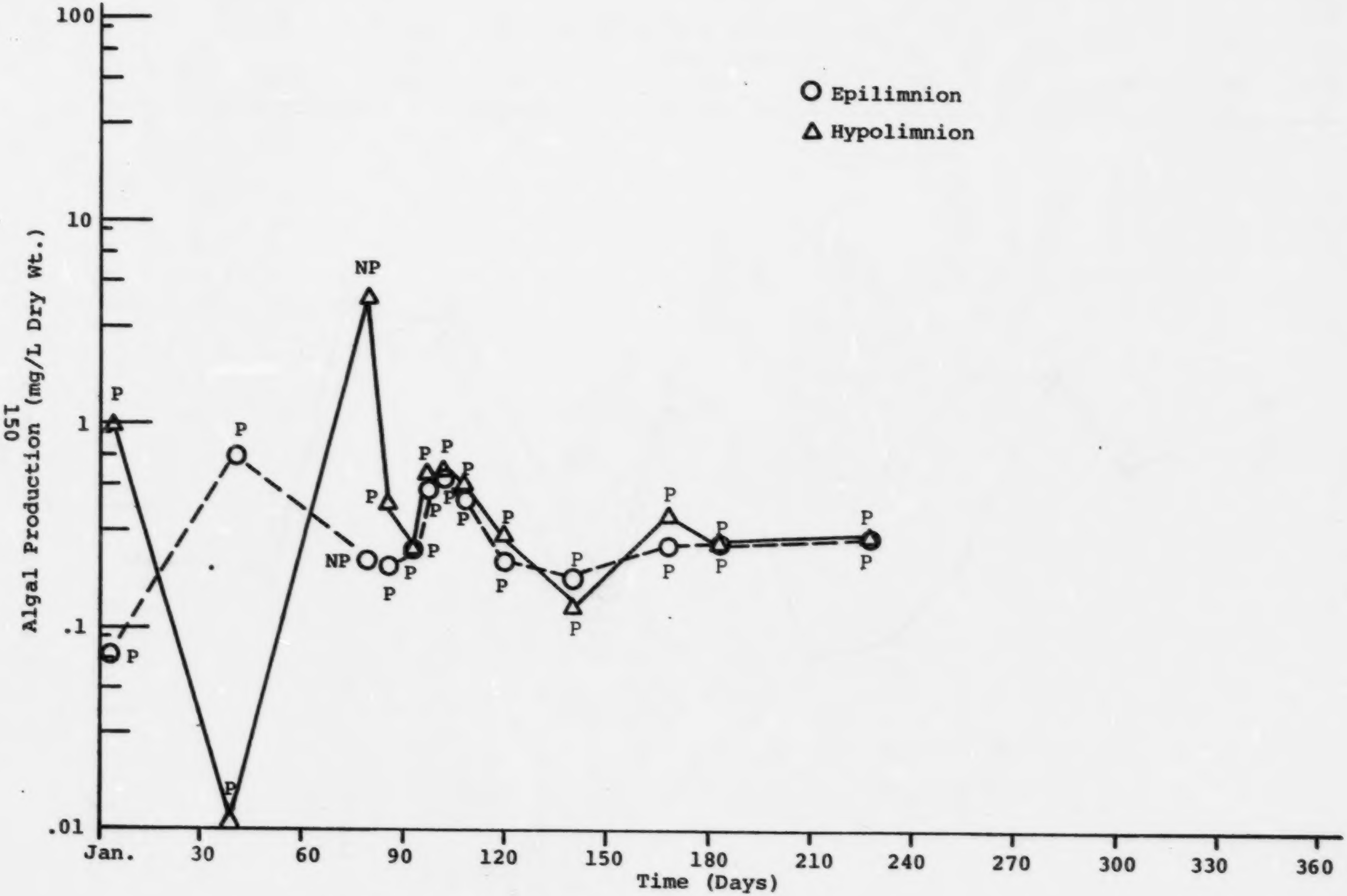

Fig. 46. Algal Production - Pickerel Lake, 1973

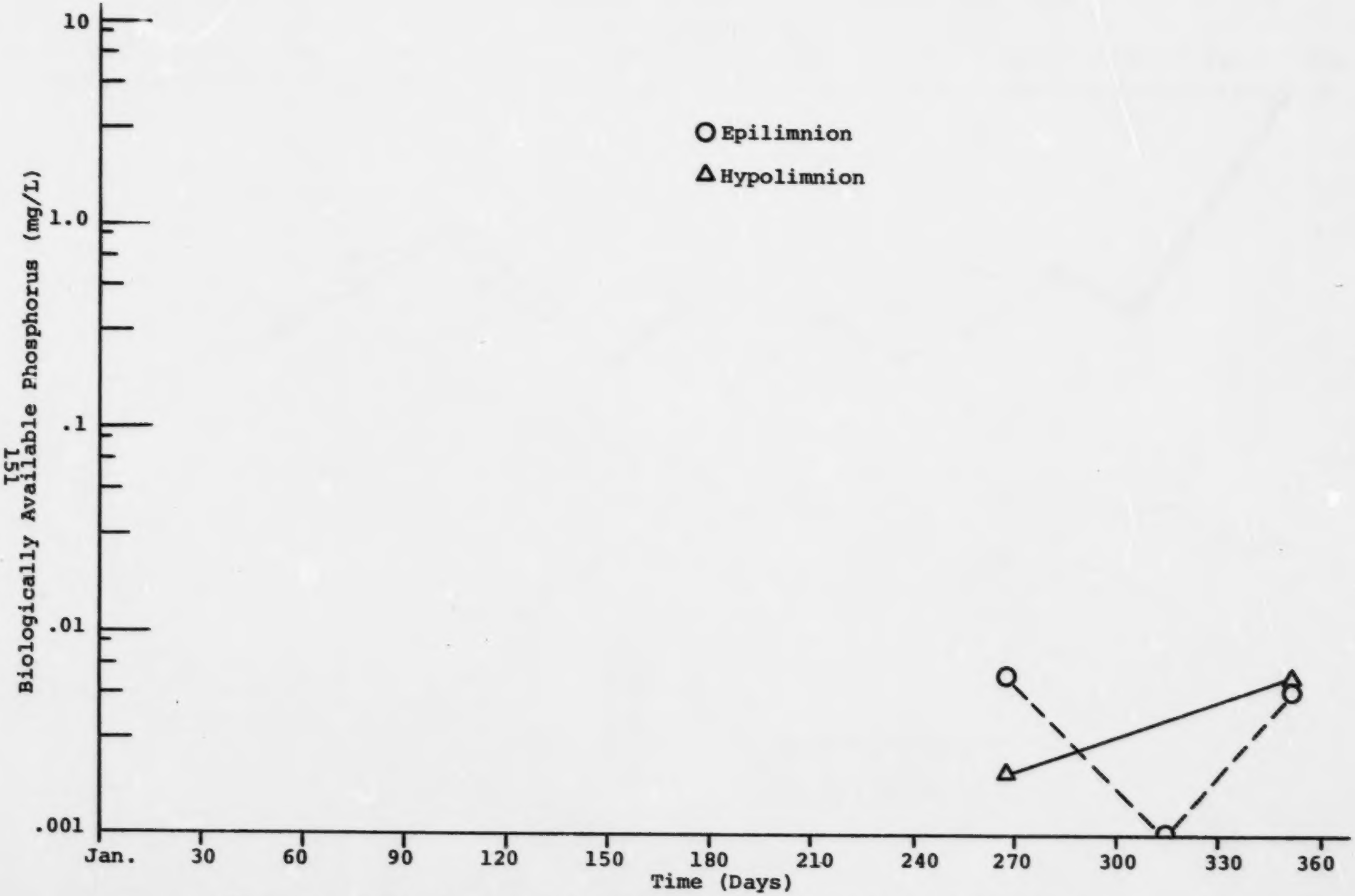

Fig. 47. Biologically Available Phosphorus - Pickerel Lake, 1971

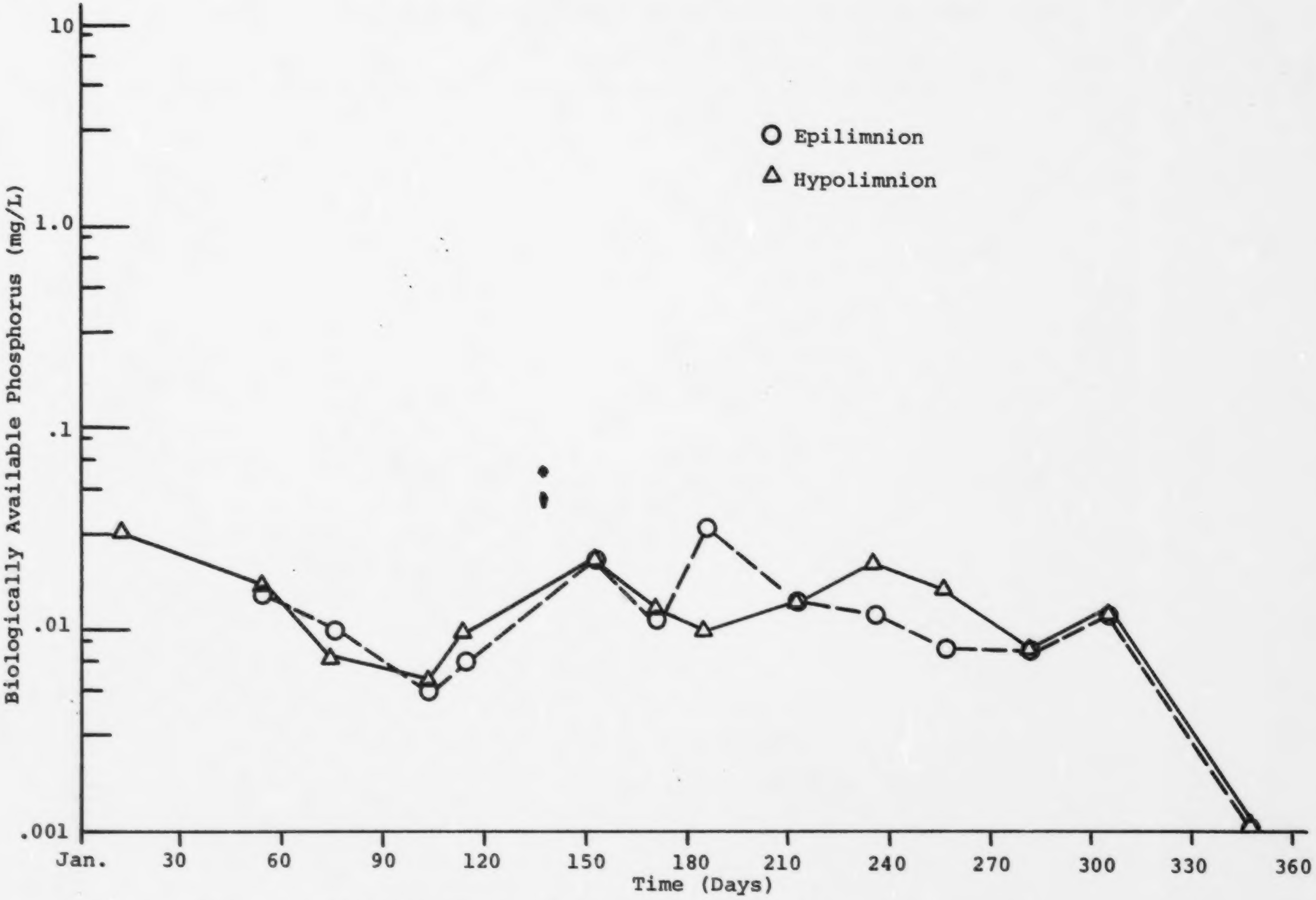

Fig. 48. Biologically Available Phosphorus - Pickerel Lake, 1972

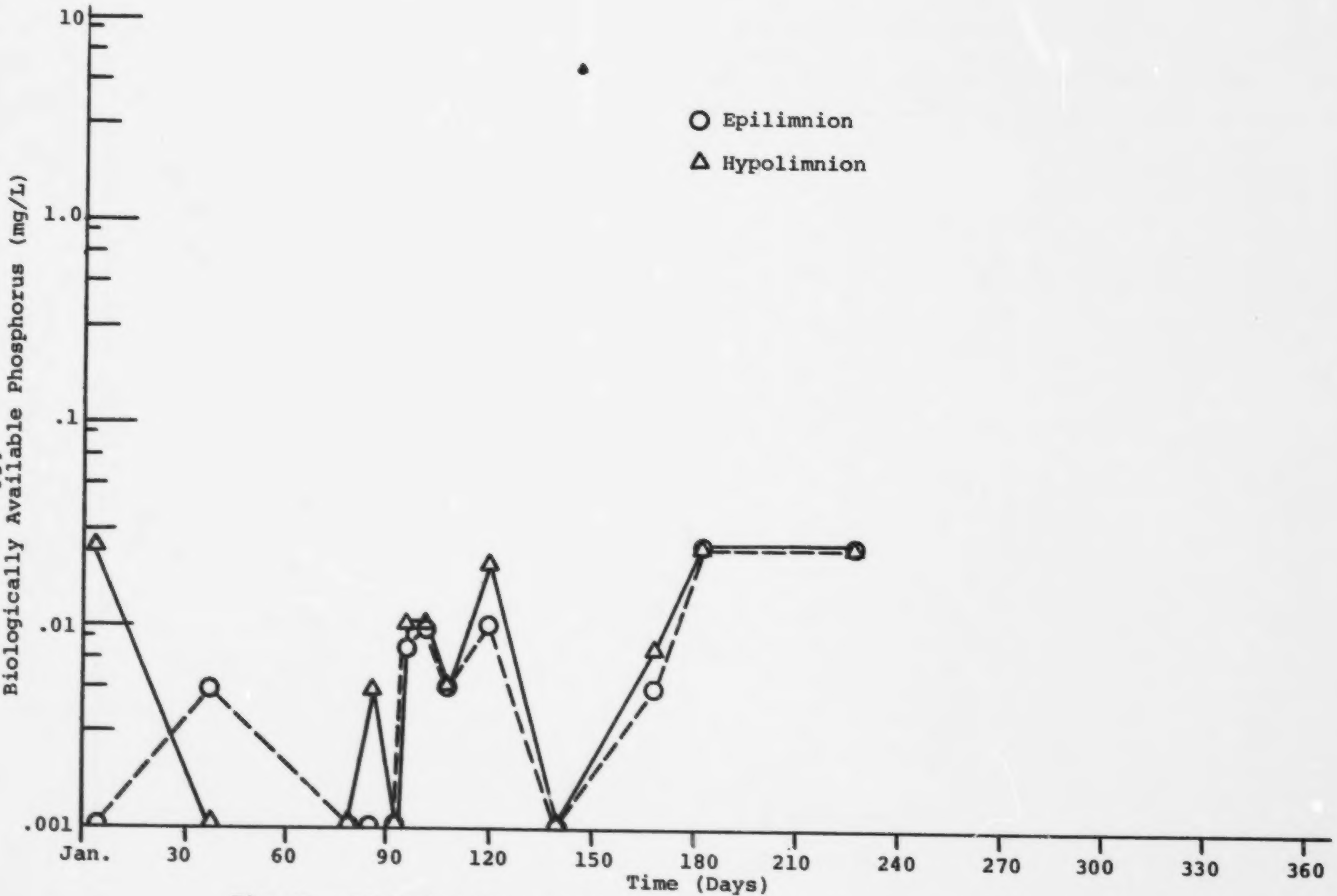

Fig. 49. Biologically Available Phosphorus - Pickerel Lake, 1973

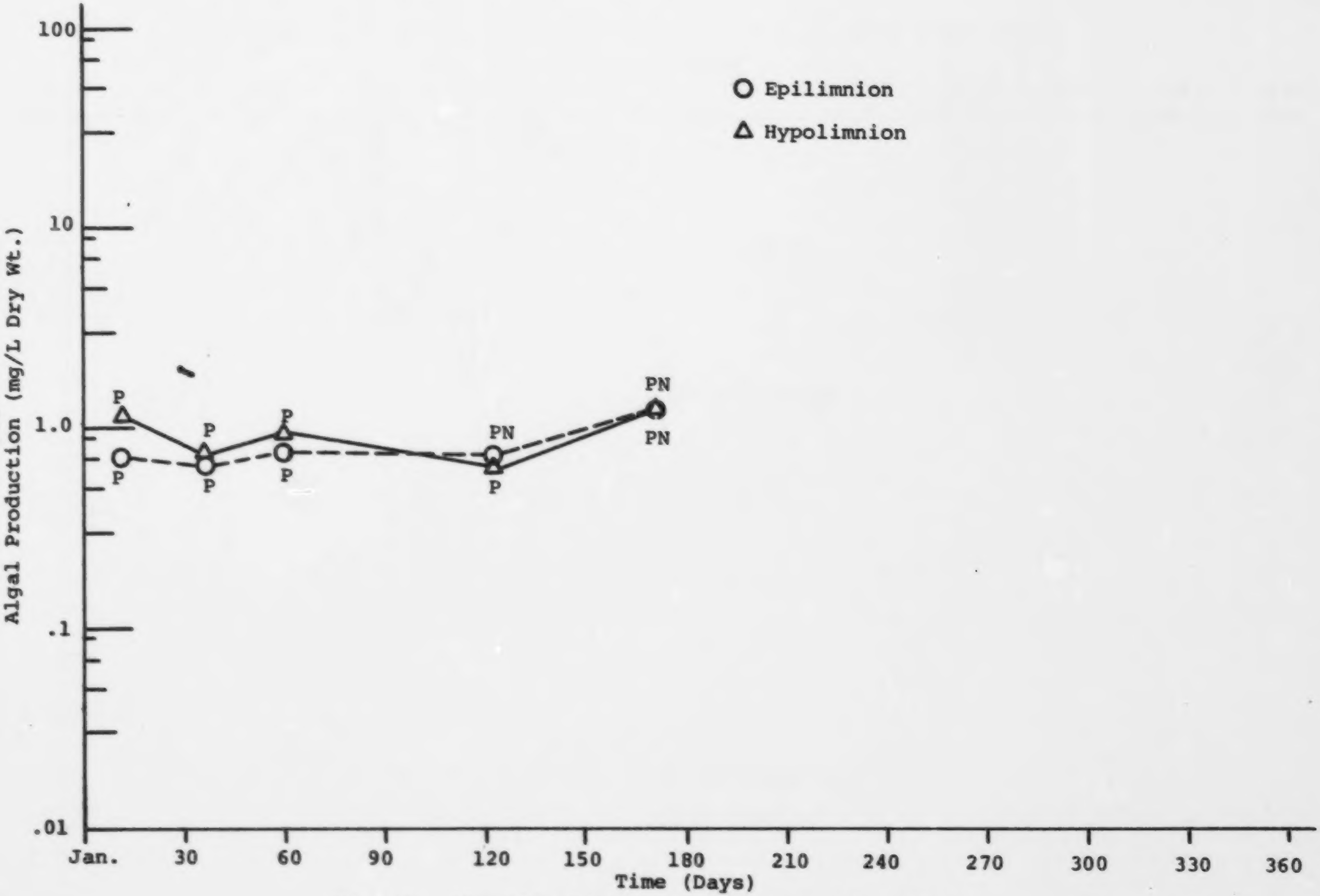

Fig. 50. Algal Production - Mayflower Lake, 1973

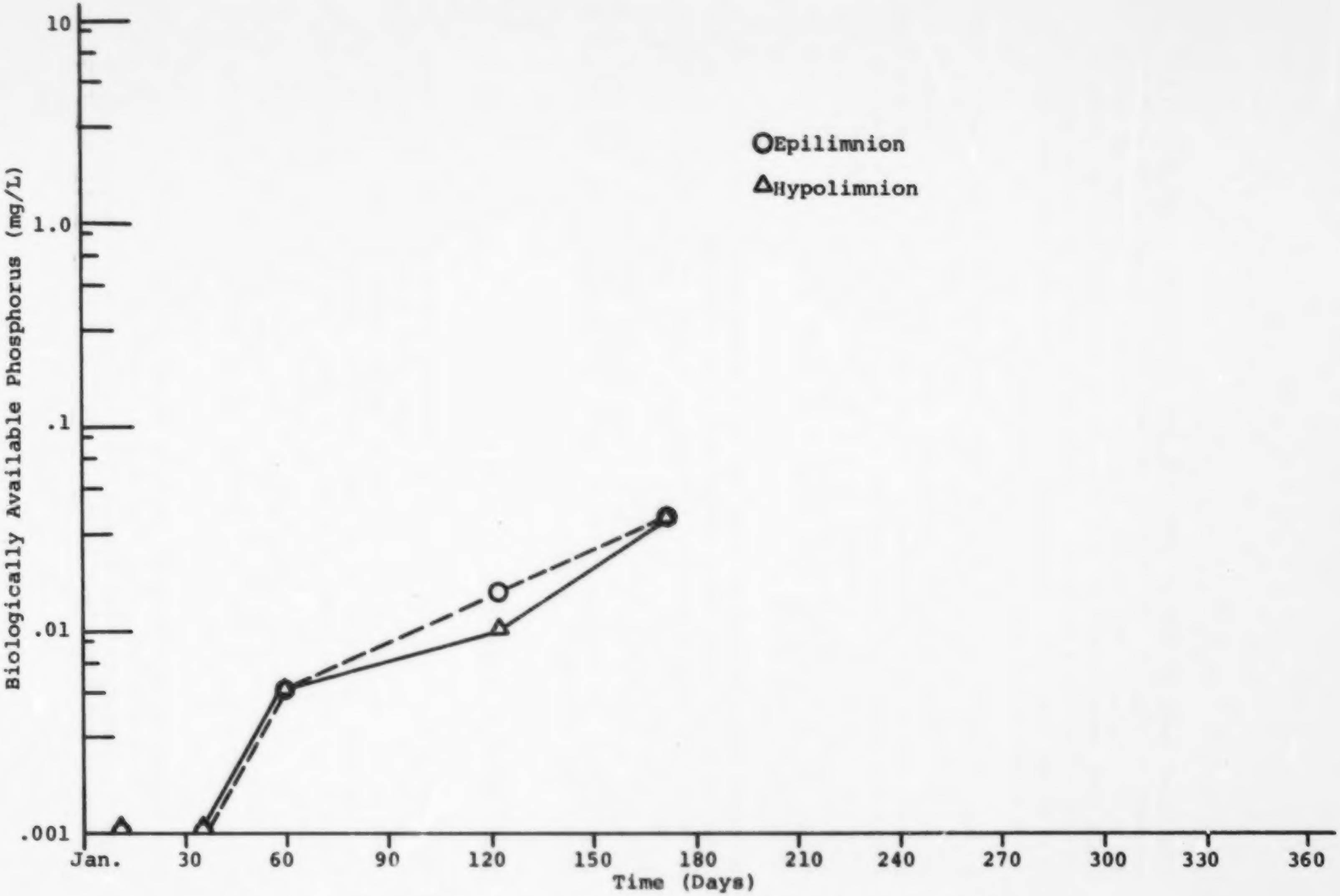

Fig. 51. Biologically Available Phosphorus, Mayflower Lake, 1973

The dual set of assay data--north and south basins--is included to depict the degree of overall variability inherent in the sampling and algal assays. The two basins of Snake Lake are very similar and would not be expected to respond differently to treatment. This similarity is reflected in the assay results. Seasonal trends are the same and absolute quantities are in good agreement.

Algal production in samples from Long and Pickerel Lakes was consistently much lower than for any of the other lakes studied. Most samples produced less than 0.5 mg/l, both before and after treatment. Algal production in samples from Pickerel Lake did not exceed 0.5 mg/l during the summer following treatment, whereas in 1972, prior to treatment, three hypolimnetic samples produced 5 mg/l. However, except for these three samples, the remaining pre- and post-treatment data were very similar.

Biomass was generally limited by phosphorus in samples from both Pickerel and Long Lakes prior to treatment, so no shift in the limiting nutrient was anticipated as a result of the aluminum treatment. Post-treatment data showed this to be the case; nitrogen was identified as limiting in only one of 97 samples from these lakes. The assay results alone are not sufficient to document the effect of treatment on the general fertility of these lakes.

Hypolimnetic aeration -

The technique of aerating the hypolimnetic waters of lakes without destruction of the thermocline appears to be a viable method of improving the recreational value of many lakes. It has been demonstrated that aerobic conditions can be maintained in the bottom waters, and when the integrity of the thermocline is retained, it is possible in some cases to provide conditions satisfactory for cold-water fisheries. In addition, the flux of nutrients from sediments to the overlying waters may be reduced as a result of the induced aerobic conditions.

The aeration units used in the study lakes consisted of three major components: a riser stack, separator box, and return pipes. The riser section used was approximately 0.5 m in diameter and extended from the bottom to the lake surface.

Air was injected at the bottom of the riser which "pumped" water to the surface and increased the dissolved oxygen content during ascent. A separator box was attached to the riser section at the lake surface. Bubbles were allowed to escape to the atmosphere at the separator, and the aerated water was then returned to the hypolimnion through flexible pipes.

Lakes receiving hypolimnetic aeration -

Mirror Lake - Waupaca County - Mirror Lake is a drainage lake of low color and moderate fertility. It has a surface area of 5.3 ha and a maximum depth of 13 m. What was once an excellent trout fishery has been compromised by an anoxic hypolimnion created by storm sewer input. The lake's basin is well sheltered, consequently spring and/or fall mixing may be ineffectual or even absent during some years. The renewal approach consists of hypolimnetic aeration during summer and winter, coupled with total mixing to extend and amplify periods of overturn in spring and fall. The equipment was installed and tested in the fall of 1972, and hypolimnetic aeration began in January 1973.

Larson Lake - Lincoln County - Larson Lake is a small, brown-water seepage lake of moderate to low fertility. It has a surface area of 5 ha and a maximum depth of 9 m. The shoreline is partly bog-like in nature, but there is no appreciable mat on the lake's surface. Available data indicate that Larson Lake seldom, if ever, mixes thoroughly in either spring or fall. The extensive hypolimnion is generally without measurable dissolved oxygen. Hypolimnetic aeration began on February 2, 1973.

Results of algal assays on samples from Mirror Lake are plotted in Figures 52 through 57 and are summarized, by season, in Table 40. These data provide a general measure of changes in lake fertility, but are not sufficient to detect short-term effects induced by the intermittent periods of aeration.

Samples from the epilimnion of Mirror Lake consistently produced more algal biomass in 1973 than in 1972. The increase was greatest during the period from June through August. However, the increase in algal production in epilimnetic samples was small in comparison to the decrease in production

Table 40. AVERAGE ALGAL BIOMASS PRODUCED IN SAMPLES FROM LAKES SUBJECTED TO HYPOLIMNETIC AERATION[a]

		Epilimnion				Hypolimnion			
Lake	Year	Dec-Feb	Mar-May	June-Aug	Sept-Nov	Dec-Feb	Mar-May	June-Aug	Sept-Nov
Mirror[b]	1971				1.6				23.
	1972	5.1	3.0	0.15	5.4	307.	138.	127.	47.
	1973	9.1	5.5	4.5		3.1	8.8	16.	
Larson[c]	1971								
	1972	2.6	0.69	1.1	2.0	1.5	0.76	9.0	7.2
	1973	3.6	1.5	1.4		15.	4.1	13.	

[a] All values given in mg/l dry weight.

[b] Aeration schedule - 8/10/72-9/8/72, hypo only; 9/19/72-11/21/72, total lake; 1/30/73-3/8/73, hypo only; 3/30/73-4/22/73, total lake; 6/21/73-8/28/73, hypo only.

[c] Aeration schedule - 2/2/73-3/16/73, hypo only; 4/25/73-9/20/73, hypo only.

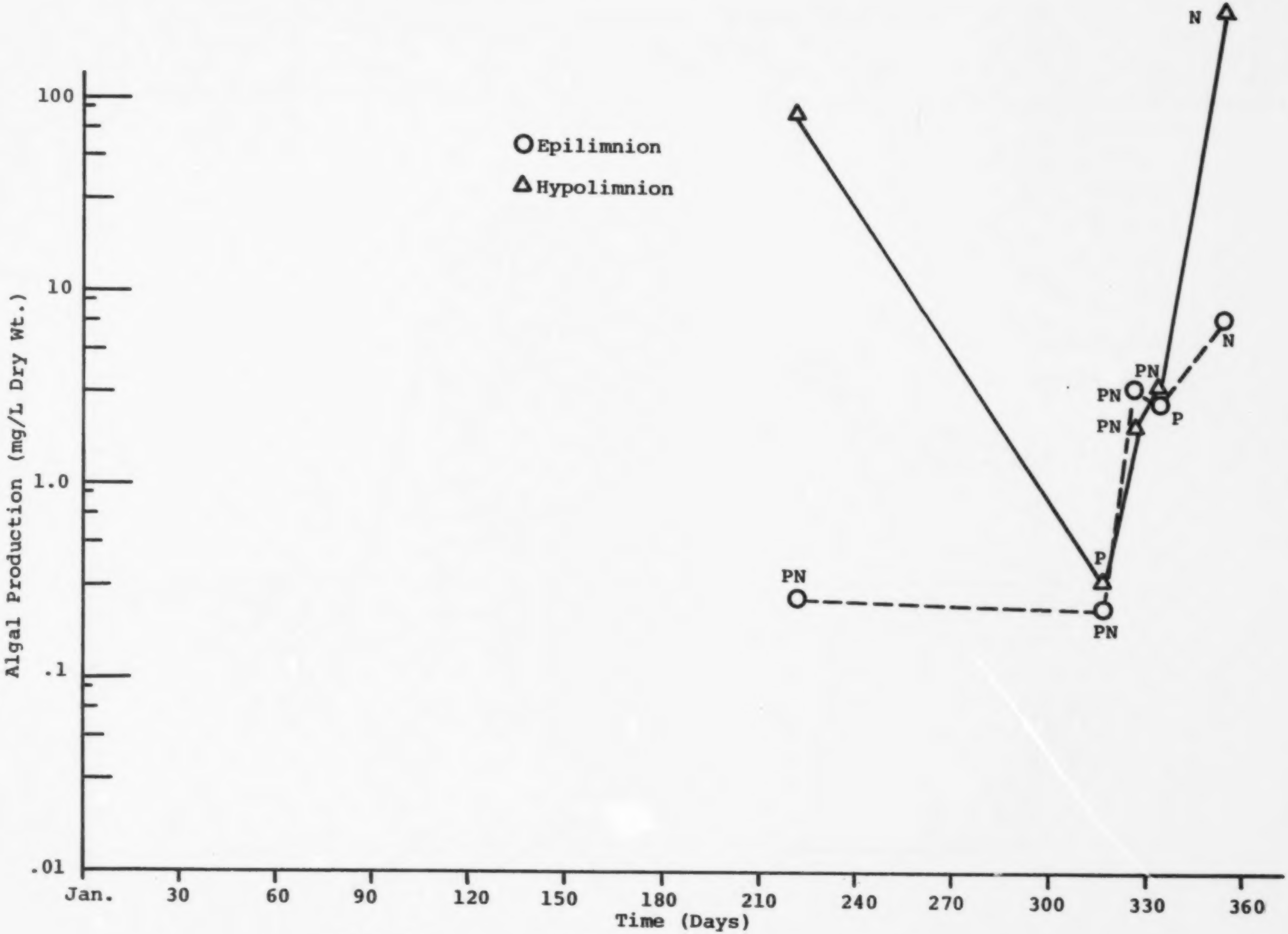

Fig. 52. Algal Production - Mirror Lake, 1971

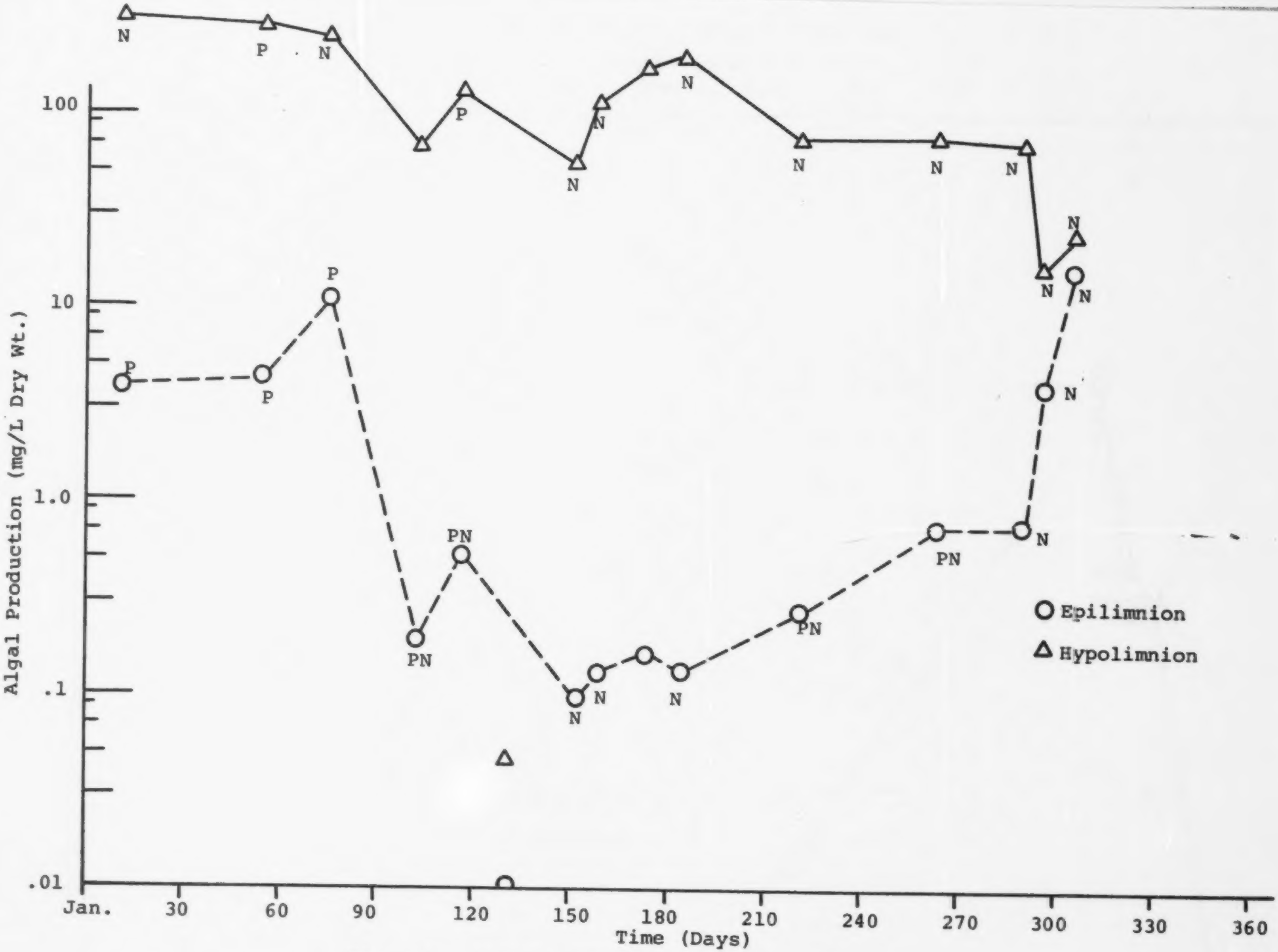

Fig. 53. Algal Production - Mirror Lake, 1972

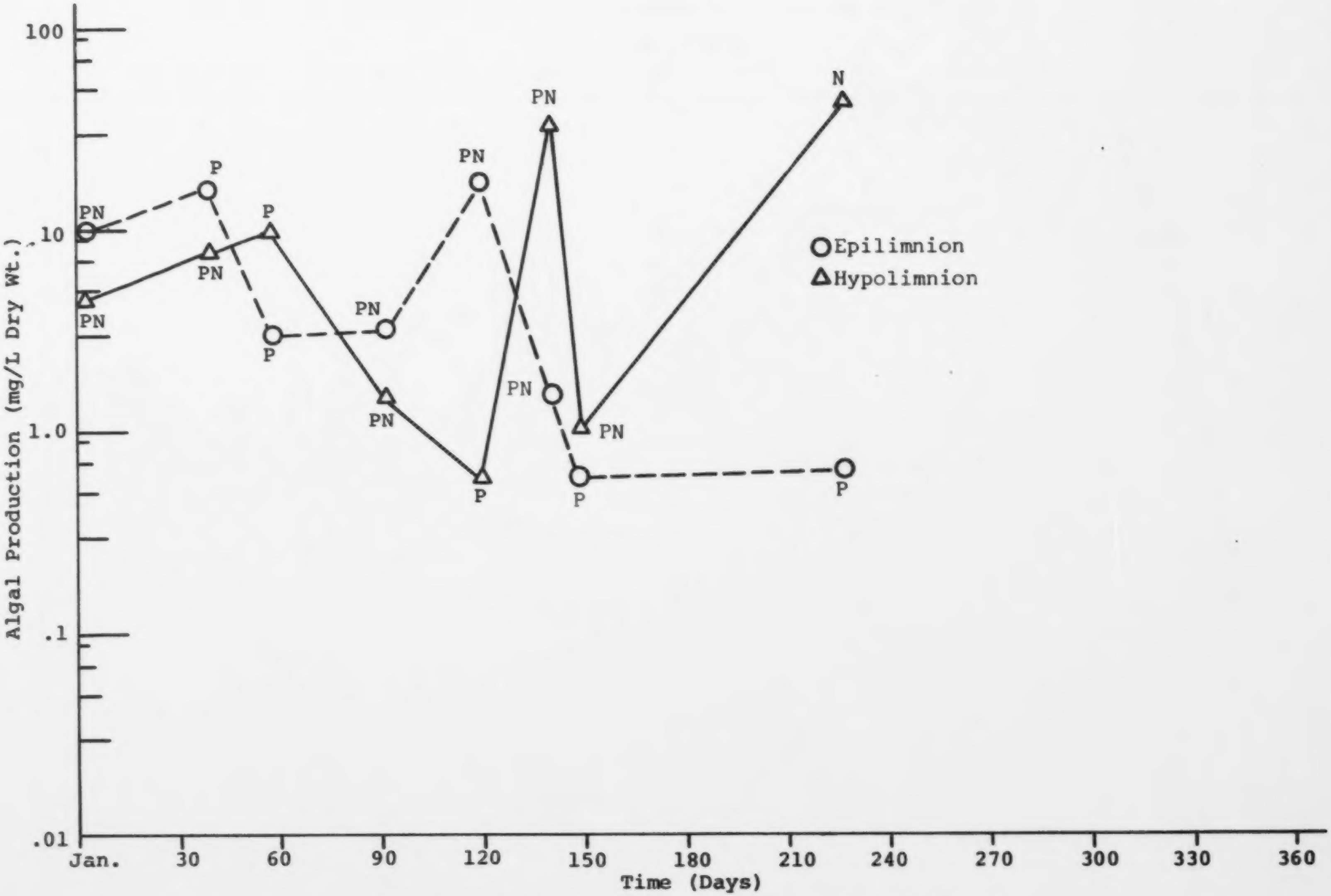

Fig. 54. Algal Production - Mirror Lake, 1973

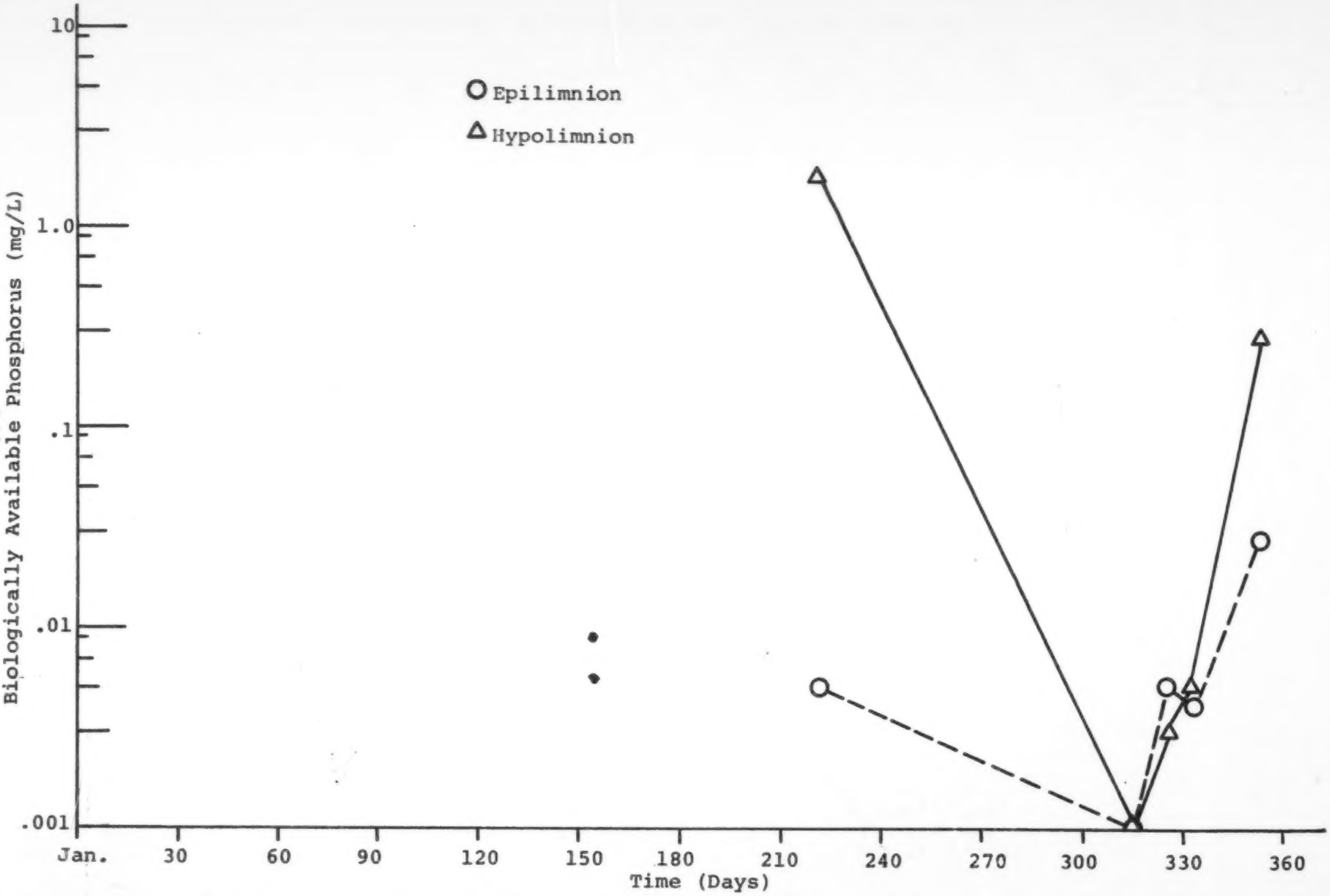

Fig. 55. Biologically Available Phosphorus - Mirror Lake, 1971

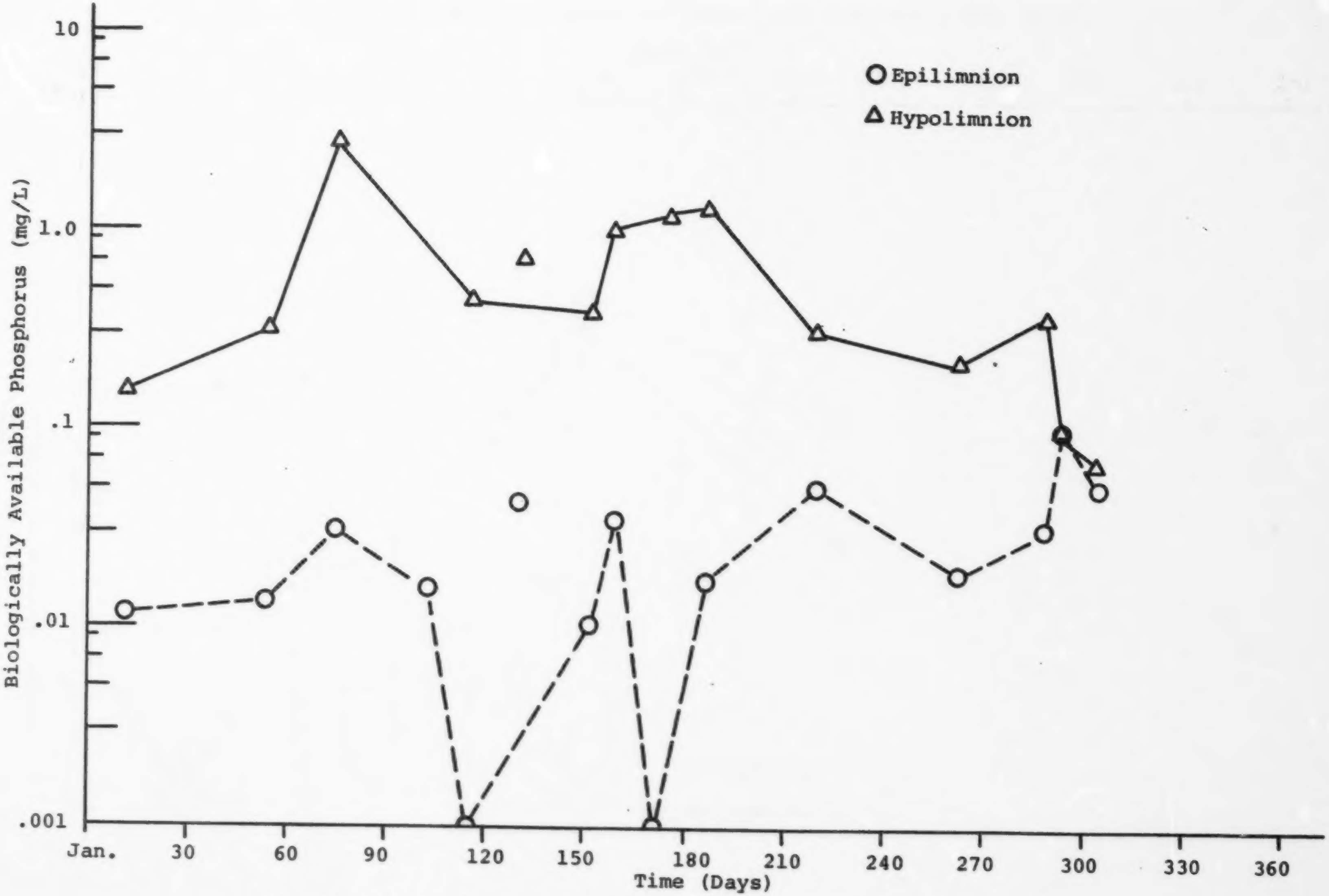

Fig. 56. Biologically Available Phosphorus - Mirror Lake, 1972

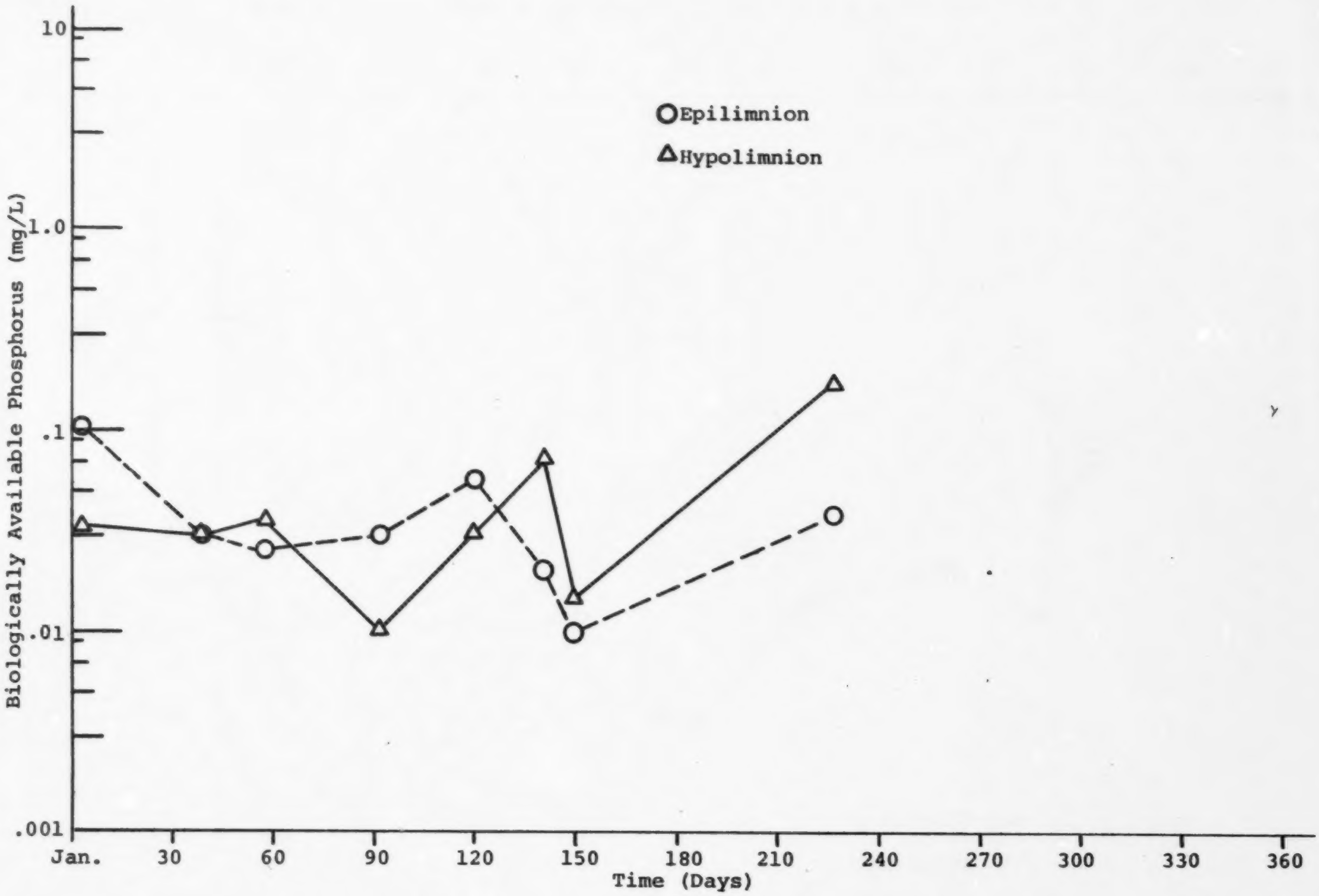

Fig. 57. Biologically Available Phosphorus - Mirror Lake, 1973

in samples from the hypolimnion. Whereas the production in epilimnetic samples in 1973 was about twice as large as in 1972, hypolimnetic samples in 1972 yielded more than ten times as much algae as the 1973 samples.

Nitrogen was identified as the nutrient which most frequently limited cell production in the 1972 samples. Nitrogen limitation was particularly prevalent in the hypolimnion samples in which 10 of 12 determinations showed a significant response to an addition of nitrogen alone. It should be noted that these results indicate that large amounts of phosphorus were available; nitrogen was in short supply only in relative terms. Sufficient nitrogen was available to permit biomass production in excess of 100 mg/l. However, in 1973, nitrogen limitation was detected on only one occasion, and algal production and biologically available phosphorus were much lower in the hypolimnetic samples.

Bioassay data for Larson Lake (Figures 58-63 , Tables 40-41) showed a lesser effect of aeration than was noted in Mirror Lake, however, only about 6 months of comparable pre- and post-treatment data were obtained. A slight increase in cell production in both the epilimnion and hypolimnion occurred in 1973 as compared to 1972. The shift from nitrogen-limited to phosphorus-limited samples, noted in Mirror Lake, also occurred in Larson Lake, but the effect was less pronounced.

Miscellaneous Studies

Drawdown and winter aeration -

Horsehead Lake - Oneida County - A program of baseline data collection was initiated on Horsehead Lake (144 ha, 3.7 m maximum depth). This lake has experienced water quality problems relating to both weeds and algae, and a combined treatment of drawdown for sediment consolidation coupled with winter aeration is contemplated for the future. Tentative treatment dates have not been scheduled. Algal assays were included as part of the baseline data collection program.

As shown in Table 42, a total of 12 assays were conducted on Windfall, White, and Shadow Lakes. These were conducted as part of short-term assessments of aquatic plant control (Windfall), urban runoff studies (Shadow), and winter aeration (White). No further work was undertaken in connection

Table 41. NUTRIENT LIMITATION IN SAMPLES FROM LAKES SUBJECTED TO HYPOLIMNETIC AERATION

Lake	Year	Location	Number of samples which responded to additions of: N	P	N&P	Total samples
Mirror	1971	epi	1	1	3	5
		hypo	1	1	2	5
	1972	epi	6	3	4	14
		hypo	10	2	-	14
	1973	epi	-	4	4	8
		hypo	1	2	5	8
Larson	1972	epi	-	2	9	11
		hypo	6	-	5	11
	1973	epi	-	10	11	22
		hypo	2	7	12	21

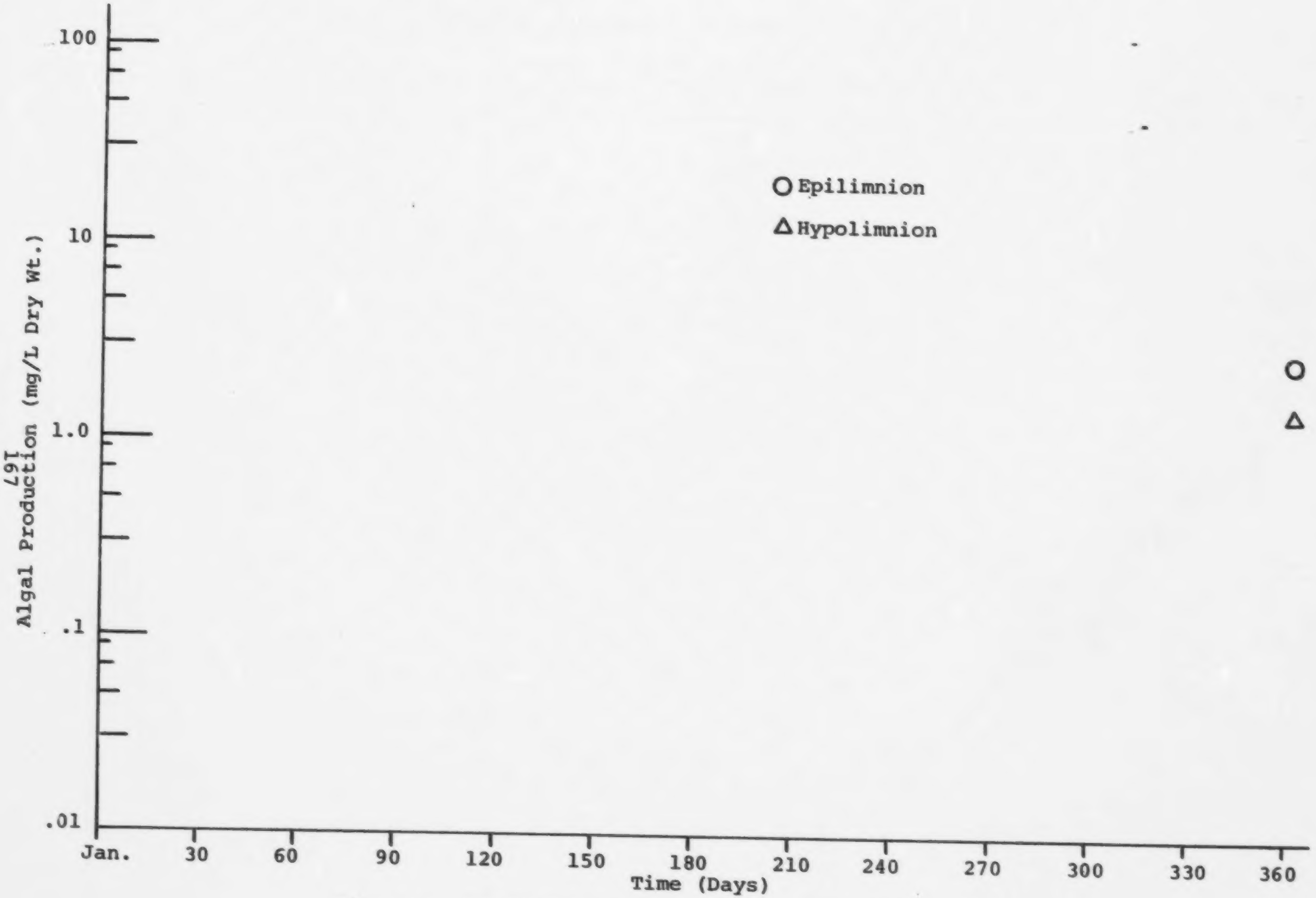

Fig. 58. Algal Production - Larson Lake, 1971

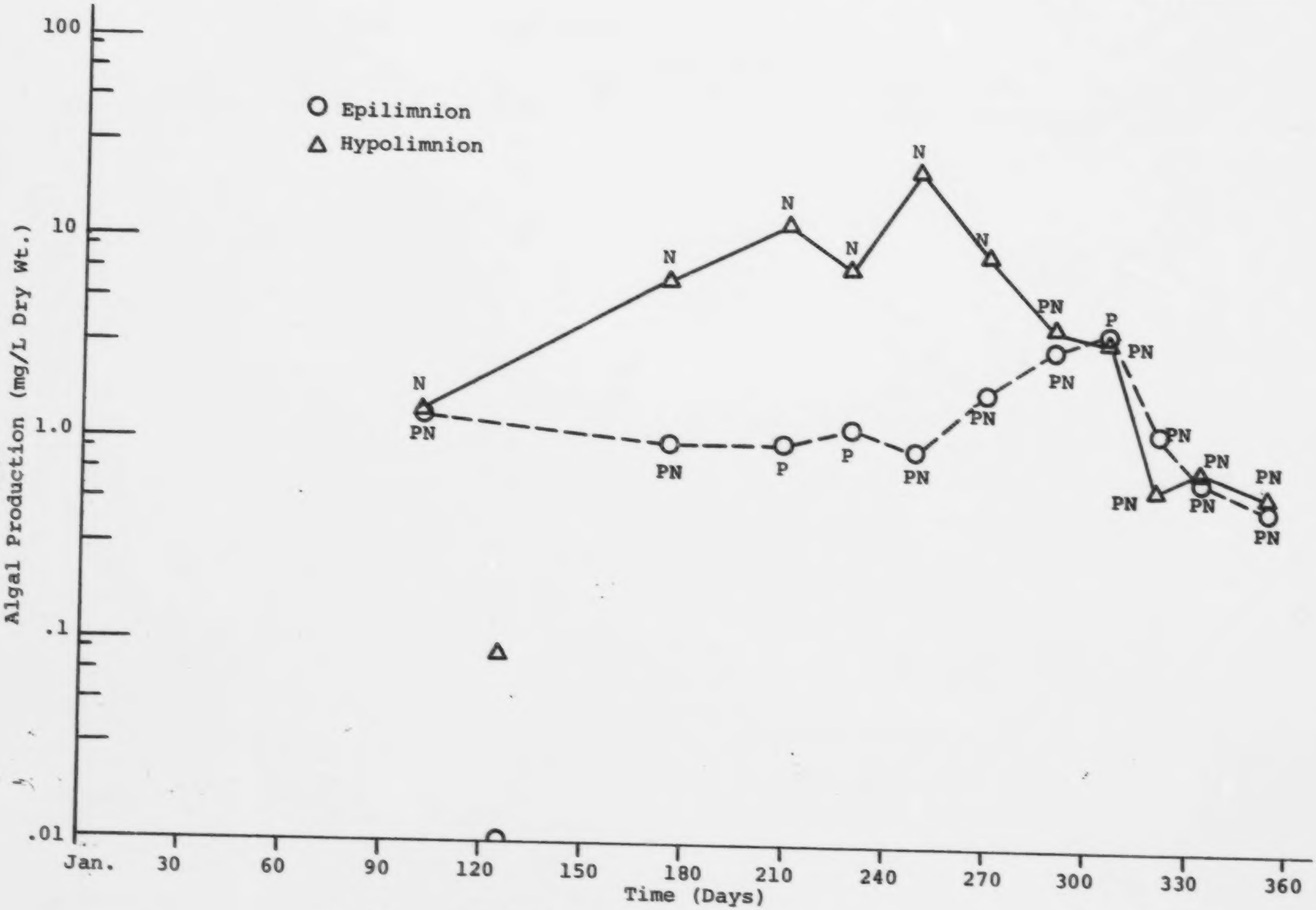

Fig. 59. Algal Production - Larson Lake, 1972

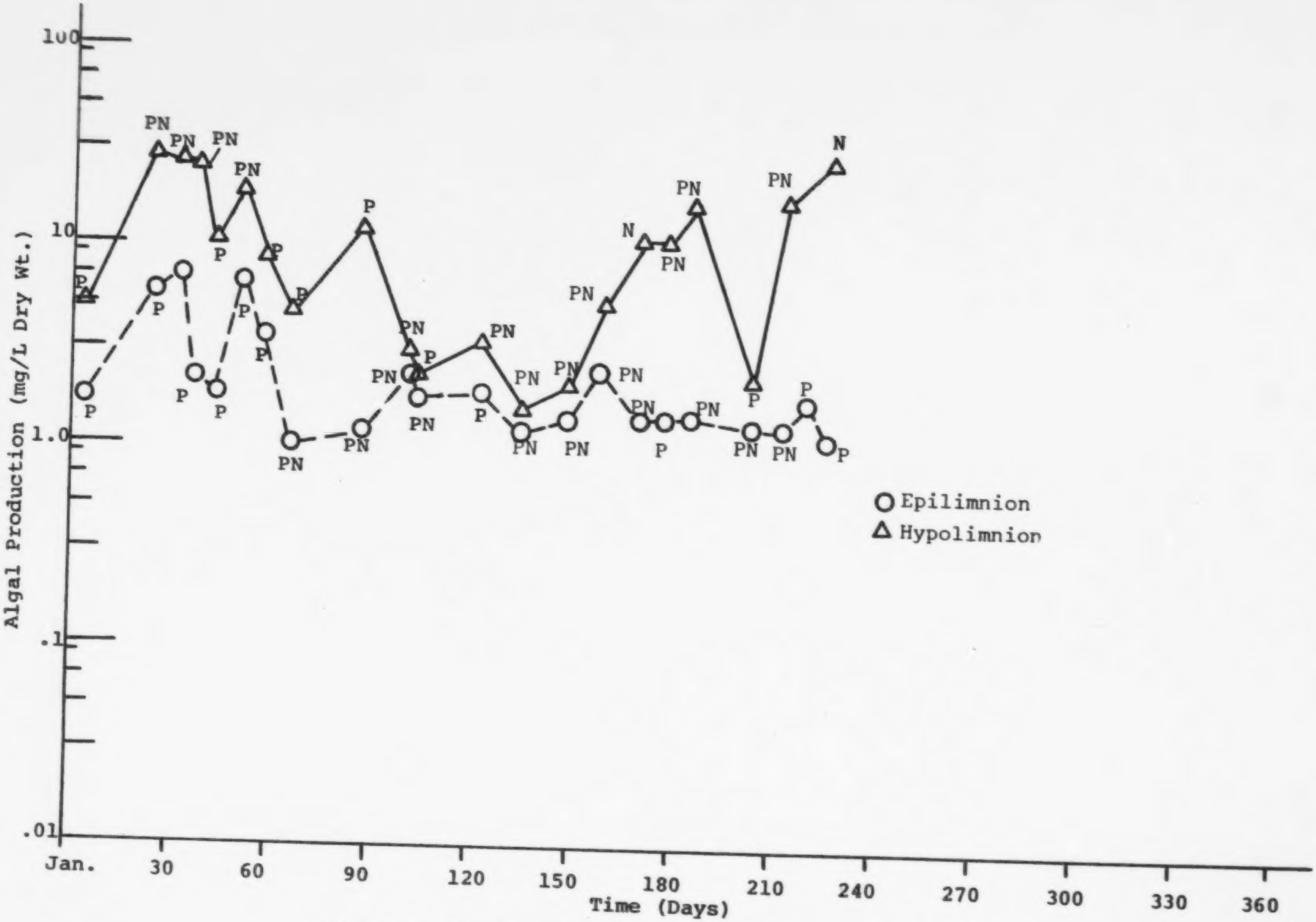

Fig.60. Algal Production - Larson Lake, 1973

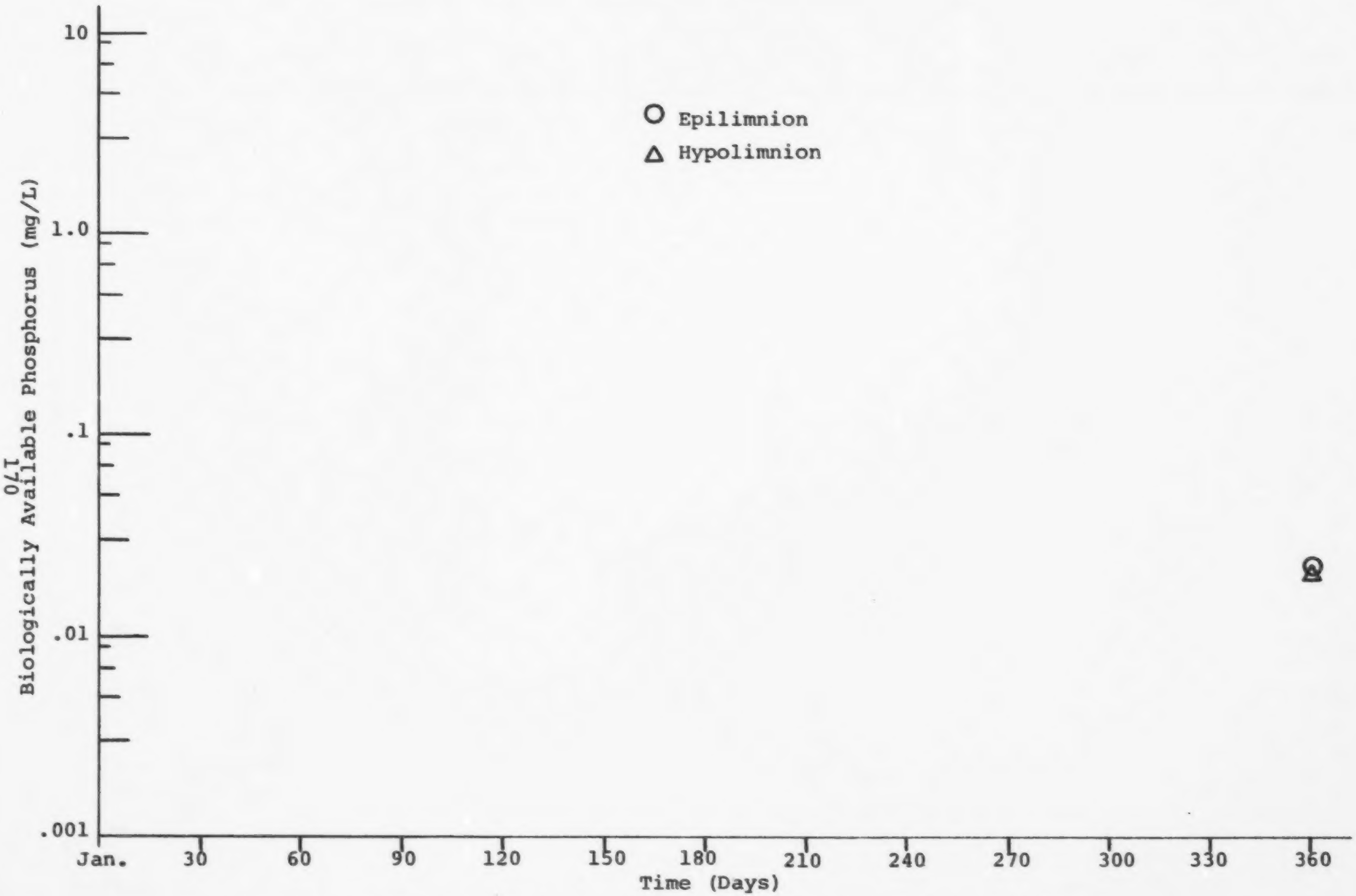

Fig. 61. Biologically Available Phosphorus - Larson Lake, 1971

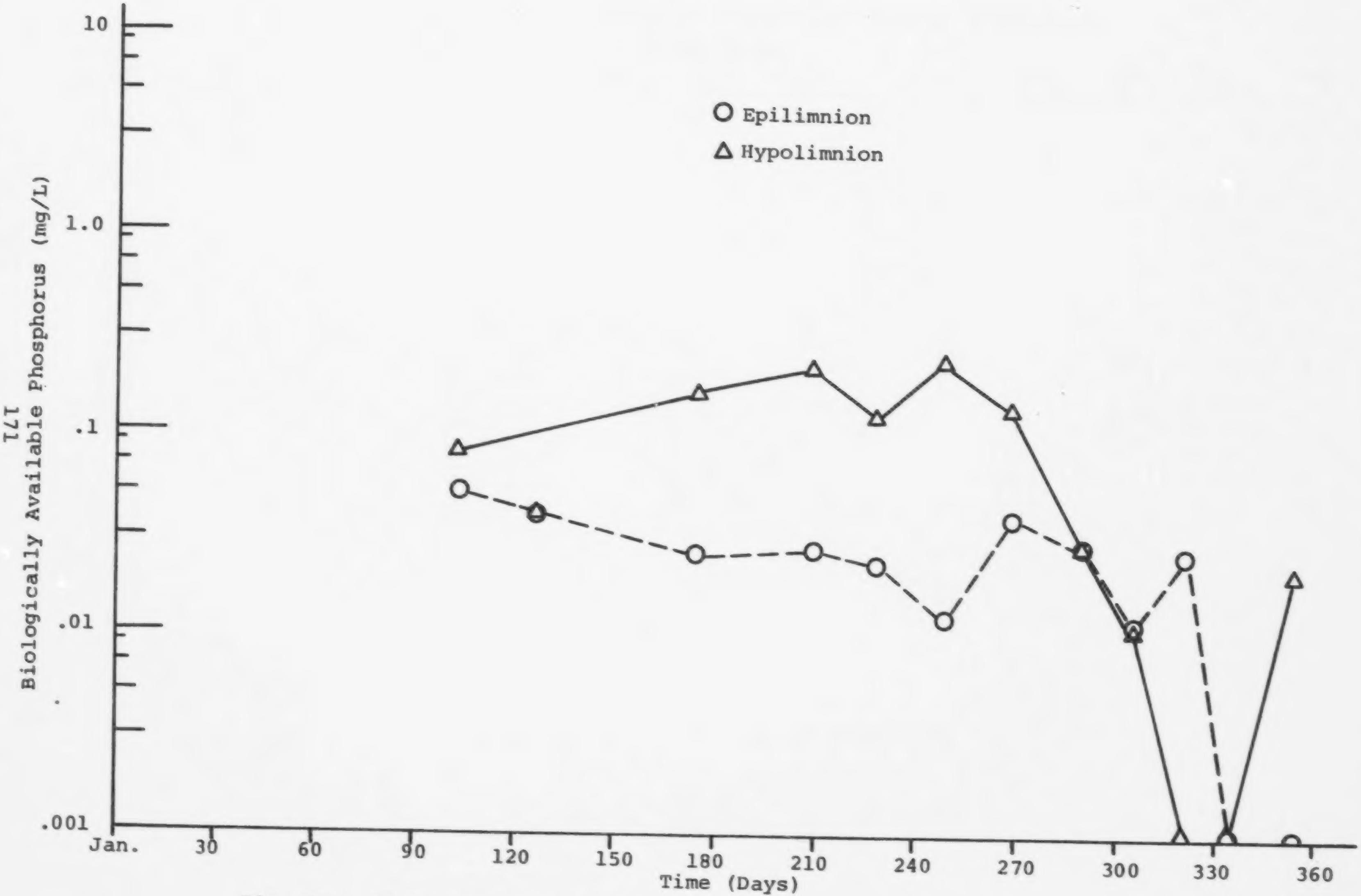

Fig. 62. Biologically Available Phosphorus - Larson Lake, 1972

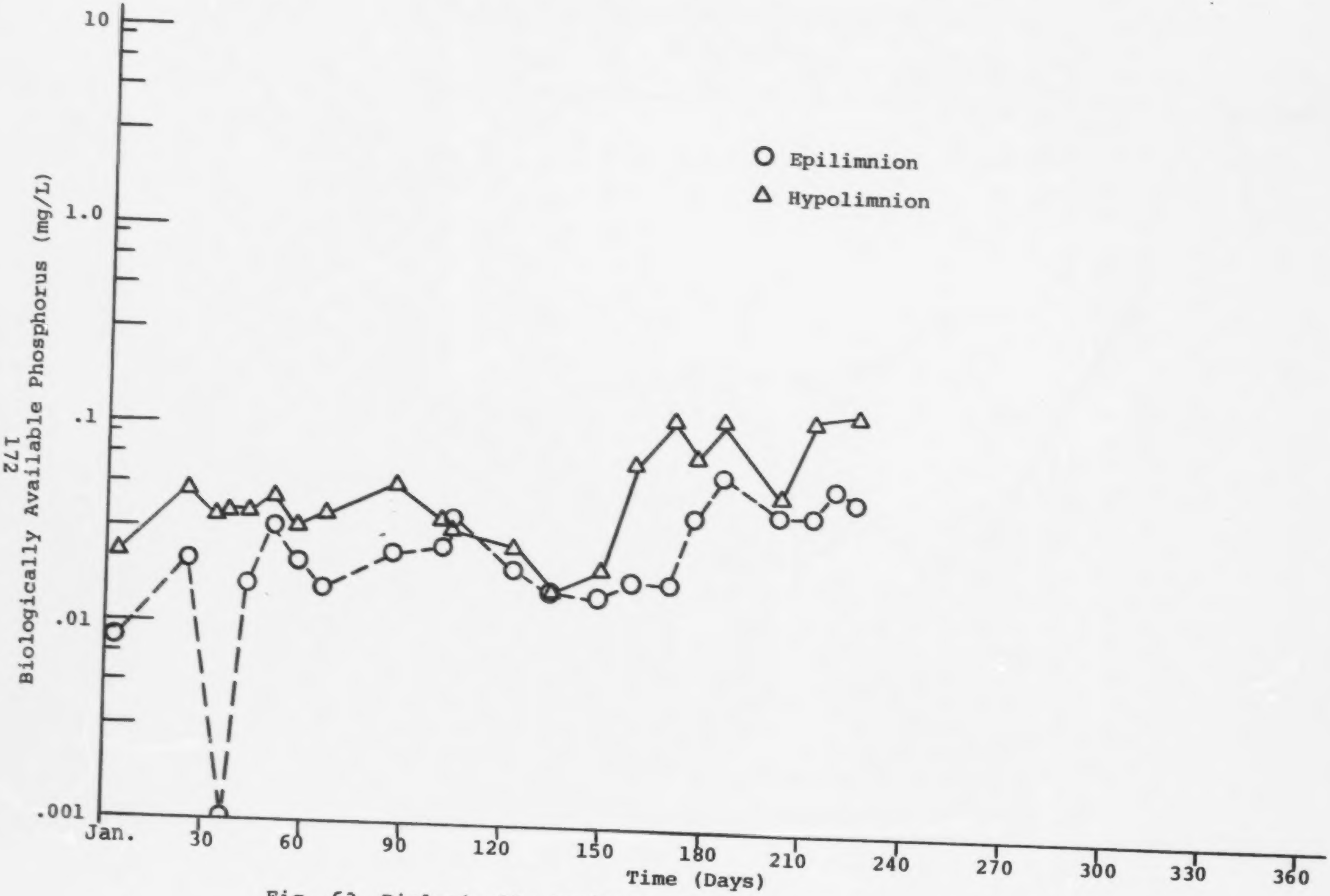

Fig. 63. Biologically Available Phosphorus - Larson Lake, 1973

Table 42. MISCELLANEOUS BIOASSAY RESULTS

Lake	Date	Depth in m	Soluble ortho-P mg/l	Biologically avail P mg/l	Biomass mg/l	Limiting Nutrient (P or N)
Shadow	11/12/71	2	-	0.001	1.7	P
		8	-	0	0.7	P
	11/22/71	2	0	0.003	1.7	P
		8	0	0.002	1.5	P
	11/30/71	2	-	0.002	1.4	P
		8	-	0.003	1.6	P
	12/21/71	2	0	0	1.0	P
		8	0	0	1.2	P
	2/24/72	2	0.032	0.009	1.0	P
		8	0.036	0.007	1.2	P
	5/11/72	2	0	0	0	P
		8	0	0	0	P
Windfall N	12/22/71	2	0.03	0.013	2.9	P
	2/2/72	1	0.009	0.018	1.8	P
		5	0.06	0.012	2.3	?
	5/5/72	1	0	0	0.06	-
		4	0	0.12	8.5	-
Windfall S	12/22/71	2	0.038	0.029	9.2	P
	2/2/72	1	0.026	0.021	1.8	P
		4	0.11	0.068	20.	N
White	1/7/73	.5	0.005	0	0.4	P
		2.5	0.016	0	1.8	P
Horsehead	2/7/73	1	0.023	0	0.3	P
	3/2/73	1	0.01	0	0	P
	5/3/73	1	0.029	0.005	0.3	-
	8/8/73	1	0.055	0.04	0.9	P

with any of these lakes. Table 42 gives the biologically available P concentration, the limiting nutrient and the biomass produced for each lake sample.

Horseshoe Lake inlets -

Because phosphorus levels in Horseshoe Lake seemed to be increasing during the early spring months of 1972, a series of water samples were taken to try to identify the source of this phosphorus. From February to August of 1973, 12 samples of inlet water were analyzed for algal growth potential (mg/l dry weight) and biologically available phosphorus. The results are given in Table 43.

Table 43. BIOASSAY RESULTS FOR SAMPLES FROM INLETS TO HORSESHOE LAKE

Site	Date	Soluble ortho-P mg/l	Biologically Avail P mg/l	Biomass mg/l
Weir	1/3/73	0.110	0.08	9
	2/7/73	0.156	0.46	74
	3/6/73	0.304	>0.2	120
	4/18/73	0.086	0.10	32
	5/23/73	0.104	0.02	23
	6/26/73	0.182	0.19	140
	7/26/73	0.151	0.07	100
Culvert	1/3/73	0.079	0.06	12
	3/6/73	0.320	>0.2	250
	5/23/73	0.104	0.04	31
	6/26/73	0.149	0.18	110
	7/26/73	0.109	0.04	66
Driveway	2/7/73	0.044	0.06	29
	4/18/73	0.151	0.16	99

The 1973 increase in productivity of Horseshoe Lake over that of 1971 and 1972 could be attributed to the high concentrations of phosphorus entering the lake throughout the entire growing season.

Comparison of phosphorus determinations -

An analysis was conducted to compare the concentrations of biologically available phosphorus as determined by the growth bioassay, to soluble orthophosphate as determined by the stannous chloride technique (Standard Methods). The chemical analyses were conducted after lake samples were autoclaved. A total of 149 paired determinations were made, and samples from each of the lakes studied were included. The results are shown in Figure 64.

Linear regression analysis indicated that the line of best fit for this data had a slope of 0.98 and an intercept of .01, indicating very nearly a one-to-one mean relationship between the two techniques. However, as shown by the plot, there was considerable variation in the data and the correlation coefficient was only 0.71. Thus, only about 50% of the variability in the biologically available phosphorus can be attributed to the orthophosphate concentration. The fact that the data cluster about a line with a slope very near 1.0 lends further support to the use of orthophosphate determinations as a measure of that portion of the total phosphorus pool which is available for algal growth.

References

1. Peterson, J. O., J. P. Wall, T. L. Wirth, and S. M. Born. Eutrophication Contro: Nutrient Inactivation by Chemical Precipitation at Horseshoe Lake, Wisconsin. Wisconsin Department of Natural Resources. Madison, Wis. Technical Bulletin No. 62. 1973. 20 p.

2. Algal Assay Procedure: Bottle Test. U.S. Government Printing Office. Washington, D.C. 1972--795-146/1 Region No. 10. August 1971. 82 p.

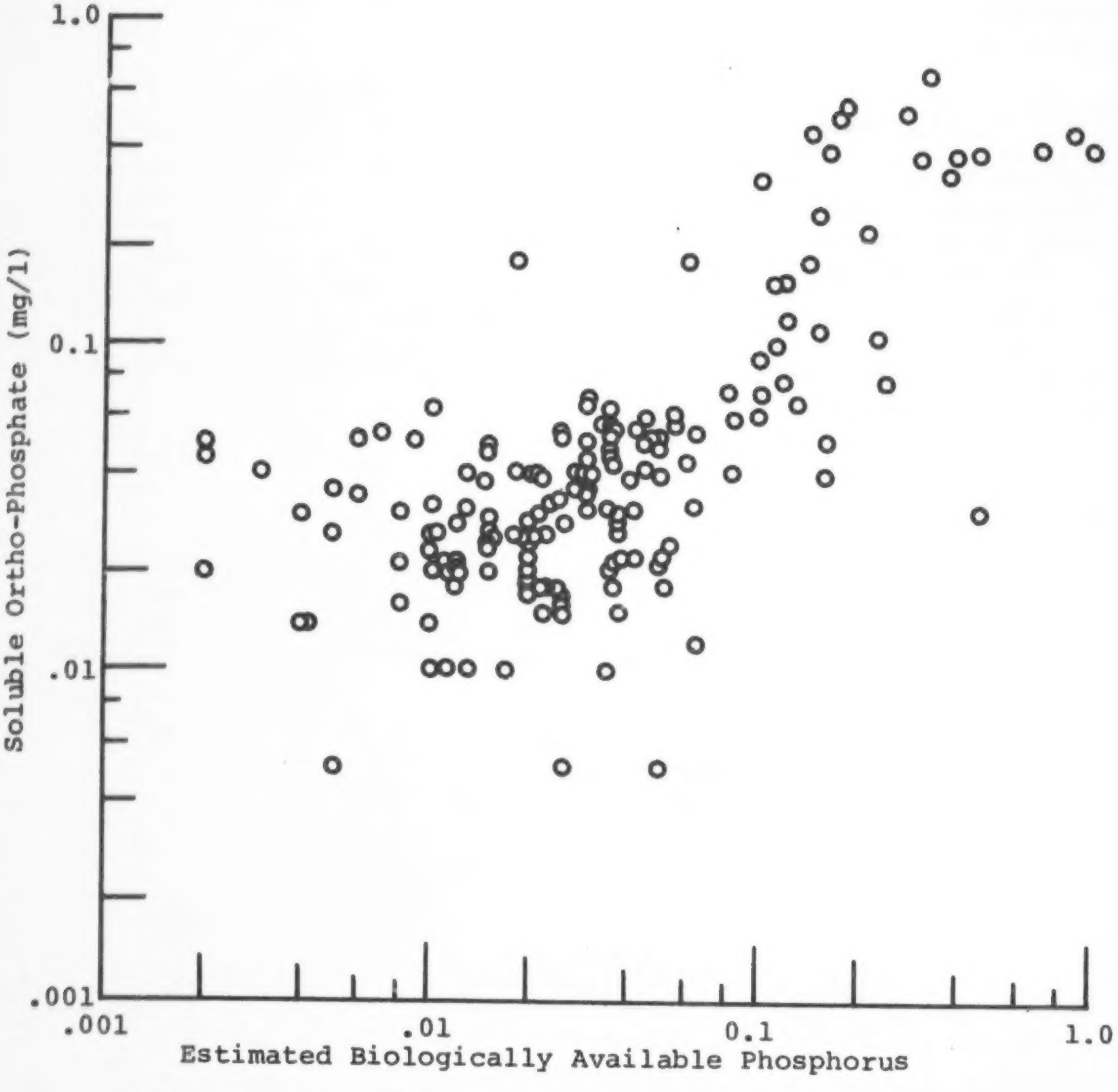

Fig. 64. Comparison of Phosphorus Determinations

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